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(54) Title: ENZYME-BASED GPROTEIN-COUPLED RECEPTOR ASSAY

(57) Abstract: Methods for detecting G-protein coupled receptor (GPCR) activity; methods of assaying GPCR activity; and methods of screening for GPCR ligands, G-protein-coupled receptor kinase (GRK) activity, and compounds that interact with components of the GPRC regulatory process are described.

TITLE OF THE INVENTION

ENZYME-BASED G PROTEIN-COUPLED RECEPTOR ASSAY

BACKGROUND OF THE INVENTION

This application claims the benefit from Provisional Application Serial No. 60/180,669, filed February 7, 2000. The entirety of that provisional application is incorporated herein by reference.

Field of the Invention

This invention relates to methods of detecting G-protein-coupled receptor (GPCR) activity, and provides methods of assaying GPCR activity and methods for screening for GPCR ligands, G-protein-coupled receptor kinase (GRK) activity, and compounds that interact with components of the GPCR regulatory process.

The actions of many extracellular signals are mediated by the interaction of G-protein-coupled receptors (GPCRs) and guanine nucleotide-binding regulatory proteins (G-proteins). G-protein-mediated signaling systems have been identified in many divergent organisms, such as mammals and yeast. The GPCRs represent a large super family of proteins which have divergent amino acid sequences, but share common structural features, in particular, the presence of seven transmembrane helical domains. GPCRs respond to, among other extracellular signals, neurotransmitters, hormones, odorants and light. Individual GPCR types activate a particular signal transduction pathway; at least ten different signal transduction pathways are known to be activated via GPCRs. For example, the beta 2-adrenergic receptor (β2AR) is a prototype mammalian GPCR. In response to agonist binding, β2AR receptors activate a G-protein (Gs) which in turn stimulates adenylate cyclase activity and results in increased cyclic adenosine monophosphate (cAMP) production in the cell.

The signaling pathway and final cellular response that result from GPCR stimulation depends on the specific class of G-protein with which the particular receptor is coupled (Hamm, "The many faces of G-Protein Signaling." J. Biol. Chem., 273:669-672 (1998)). For instance, coupling to the Gs class of G-proteins stimulates cAMP production and activation of Protein Kinase A and C pathways, whereas coupling to the Gi class of G-proteins down regulates cAMP. Other second messenger systems as calcium, phosphlipase C, and phosphatidylinositol 3 may also be utilized. As a consequence, GPCR signaling events have predominantly been measured via quantification of these second messenger products.

A common feature of GPCR physiology is desensitization and recycling of the receptor through the processes of receptor phosphorylation, endocytosis and dephosphorylation (Ferguson, et al., "G-protein-coupled receptor regulation: role of G-protein-coupled receptor kinases and arrestins." Can. J. Physiol. Pharmacol., 74:1095-1110 (1996)). Ligand-occupied GPCRs can be phosphorylated by two families of serine/threonine kinases, the G-protein-coupled receptor kinases (GRKs) and the second messenger-dependent protein kinases such as protein kinase A and protein kinase C. Phosphorylation by either class of kinases serves to down-regulate the receptor by uncoupling it from its corresponding G-protein. GRK-phosphorylation also serves to down-regulate the receptor by recruitment of a class of proteins known as the arrestins that bind the cytoplasmic domain of the receptor and promote clustering of the receptor into endocytic vescicles. Once the receptor is endocytosed, it will either be degraded in lysosomes or dephosphorylated and recycled back to the plasma membrane as fully-functional receptor.

Binding of an arrestin protein to an activated receptor has been documented as a common phenomenon for a variety of GPCRs ranging from rhodopsin to β2AR to the

neurotensin receptor (Barak, et al., "A β-arrestin/Green Fluorescent fusion protein biosensor for detecting G-Protein-Coupled Receptor Activation," J. Biol. Chem., 272:27497-500 (1997)). Consequently, monitoring arrestin interaction with a specific GPCR can be utilized as a generic tool for measuring GPCR activation. Similarly, a single G-protein and GRK also partner with a variety of receptors (Hamm, et al. (1998) and Pitcher et al., "G-Protein-Coupled Receptor Kinases," Annu. Rev. Biochem., 67:653-92 (1998)), such that these protein/protein interactions may also be monitored to determine receptor activity.

The present invention involves the use of a proprietary technology (ICAST™, Intercistronic Complementation Analysis Screening Technology) for monitoring protein/protein interactions in GPCR signaling. The method involves using two inactive β-galactosidase mutants, each of which is fused with one of two interacting protein pairs, such as a GPCR and an arrestin. The formation of an active β-galactosidase complex is driven by interaction of the target proteins. In this system, β-galactosidase activity acts as a read out of GPCR activity. FIGURE 23 is a schematic depicting the method of the present invention. FIGURE 23 shows two inactive mutants that become active when they interact. In addition, this technology could be used to monitor GPCR-mediated signaling pathways via other downstream signaling components such as G-proteins, GRKs or c-Src.

Many therapeutic drugs in use today target GPCRs, as they regulate vital physiological responses, including vasodilation, heart rate, bronchodilation, endocrine secretion and gut peristalsis. See, e.g., Lefkowitz et al., Annu. Rev. Biochem., 52:159 (1983). For instance, drugs targeting the highly studied GPCR, β2AR are used in the treatment of anaphylaxis, shock hypertension, asthma and other conditions. Some of these drugs mimic

the ligand for this receptor. Other drugs act to antagonize the receptor in cases when disease arises from spontaneous activity of the receptor.

Efforts such as the Human Genome Project are identifying new GPCRs ("orphan" receptors) whose physiological roles and ligands are unknown. It is estimated that several thousand GPCRs exist in the human genome. Of the 250 GPCRs identified to date, only 150 have been associated with ligands.

SUMMARY OF THE INVENTION

A first aspect of the present invention is a method that monitors GPCR function proximally at the site of receptor activation, thus providing more information for drug discovery purposes due to fewer competing mechanisms. Activation of the GPCR is measured by a read-out for interaction of the receptor with a regulatory component such as arrestin, G-protein, GRK or other kinases, the binding of which to the receptor is dependent upon agonist occupation of the receptor. Protein/protein interaction is detected by complementation of reporter proteins such as utilized by the ICAST™ technology.

A further aspect of the present invention is a method of assessing G-protein-coupled receptor (GPCR) pathway activity under test conditions by providing a test cell that expresses a GPCR, e.g., muscarinic, adrenergic, dopamine, angiotensin or endothelin, as a fusion protein to a mutant reporter protein and interacting, i.e., G-proteins, arrestin or GRK, as a fusion protein with a complementing reporter protein. When test cells are exposed to a known agonist to the target GPCR under test conditions, activation of the GPCR will be monitored by complementation of the reporter enzyme. Increased reporter enzyme activity reflects interaction of the GPCR with its interacting protein partner.

A further aspect of the present invention is a method of assessing GPCR pathway activity in the presence of a test kinase.

A further aspect of the present invention is a method of assessing GPCR pathway activity in the presence of a test G-protein.

A further aspect of the present invention is a method of assessing GPCR pathway activity upon exposure of the test cell to a test ligand.

A further aspect of the present invention is a method of assessing GPCR pathway activity upon co-expression in the test cell of a second receptor.

A further aspect of the present invention is a method for screening for a ligand or agonists to an orphan GPCR. The ligand or agonist could be contained in natural or synthetic libraries or mixtures or could be a physical stimulus. A test cell is provided that expresses the orphan GPCR as a fusion protein with one β-galactosidase mutant and, for example, an arrestin or mutant form of arrestin as a fusion protein with another β-galactosidase mutant. The interaction of the arrestin with the orphan GPCR upon receptor activation is measured by enzymatic activity of the complemented β-galactosidase. The test cell is exposed to a test compound, and an increase in β-galactosidase activity indicates the presence of a ligand or agonist.

A further aspect of the present invention is a method for screening a protein of interest, for example, an arrestin protein (or mutant form of the arrestin protein) for the ability to bind to a phosphorylated, or activated, GPCR. A cell is provided that expresses a GPCR and contains β-arrestin. The cell is exposed to a known GPCR agonist and then reporter enzyme activity is detected. Increased reporter enzyme activity indicates that the β-arrestin molecule can bind to phosphorylated, or activated, GPCR in the test cell.

A further aspect of the present invention is a method to screen for an agonist to a specific GPCR. The agonist could be contained in natural or synthetic libraries or could be a physical stimulus. A test cell is provided that expresses a GPCR as a fusion protein with one β-galactosidase mutant and, for example, an arrestin as a fusion protein with another β-galactosidase mutant. The interaction of arrestin with the GPCR upon receptor activation is measured by enzymatic activity of the complemented β-galactosidase. The test cell is exposed to a test compound, and an increase in β-galactosidase activity indicates the presence of an agonist. The test cell may express a known GPCR or a variety of known GPCRs, or may express an unknown GPCR or a variety of unknown GPCRs. The GPCR may be, for example, an odorant GPCR or a βAR GPCR.

A further aspect of the present invention is a method of screening a test compound for G-protein-coupled receptor (GPCR) antagonist activity. A test cell is provided that expresses a GPCR as a fusion protein with one β-galactosidase mutant and, for example, an arrestin as a fusion protein with another β-galactosidase mutant. The interaction of arrestin with the GPCR upon receptor activation is measured by enzymatic activity of the complemented β-galactosidase. The test cell is exposed to a test compound, and an increase in β-galactosidase activity indicates the presence of an agonist. The cell is exposed to a test compound and to a GPCR agonist, and reporter enzyme activity is detected. When exposure to the agonist occurs at the same time as or subsequent to exposure to the test compound, a decrease in β-galactosidase activity after exposure to the test compound indicates that the test compound has antagonist activity to the GPCR.

A further aspect of the present invention is a method of screening a sample solution for the presence of an agonist, antagonist or ligand to a G-protein-coupled receptor (GPCR).

A test cell is provided that expresses a GPCR fusion and contains, for example, a β-arrestin protein fusion. The test cell is exposed to a sample solution, and reporter enzyme activity is assessed. Changed reporter enzyme activity after exposure to the sample solution indicates the sample solution contains an agonist, antagonist or ligand for a GPCR expressed in the cell.

A further aspect of the present invention is a method of screening a cell for the presence of a G-protein-coupled receptor (GPCR).

A further aspect of the present invention is a method of screening a plurality of cells for those cells which contain a G-protein coupled receptor (GPCR).

A further aspect of the invention is a method for mapping GPCR-mediated signaling pathways. For instance, the system could be utilized to monitor interaction of c-src with β-arrestin-1 upon GPCR activation. Additionally, the system could be used to monitor protein/protein interactions involved in cross-talk between GPCR signaling pathways and other pathways such as that of the receptor tyrosine kinases or Ras/Raf.

A further aspect of the invention is a method for monitoring homo- or hetero-dimerization of GPCRs upon agonist or antagonist stimulation.

A further aspect of the invention is a method of screening a cell for the presence of a G-protein-coupled receptor (GPCR) responsive to a GPCR agonist. A cell is provided that contains protein partners that interact downstream in the GPCR's pathway. The protein partners are expressed as fusion proteins to the mutant, complementing enzyme and are used to monitor activation of the GPCR. The cell is exposed to a GPCR agonist and then enzymatic activity of the reporter enzyme is detected. Increased reporter enzyme activity indicates that the cell contains a GPCR responsive to the agonist.

The invention is achieved by using ICAST™ protein/protein interaction screening to map signaling pathways. This technology is applicable to a variety of known and unknown GPCRs with diverse functions. They include, but are not limited to, the following sub-families of GPCRs:

(a) receptors that bind to amine-like ligands-Acetylcholine muscarinic receptor (M1 to M5), alpha and beta Adrenoceptors, Dopamine receptors (D1, D2, D3 and D4), Histamine receptors (H1 and H2), Octopamine receptor and Serotonin receptors (5HT1, 5HT2, 5HT4, 5HT5, 5HT6, 5HT7);

(b) receptors that bind to a peptide ligand-Angiotensin receptor, Bombesin receptor, Bradykinin receptor, C-C chemokine receptors (CCR1 to CCR8, and CCR10), C-X-C type Chemokine receptors (CXC-R5), Cholecystokinin type A receptor, CCK type receptors, Endothelin receptor, Neurotesin receptor, FMLP-related receptors, Somatostatin receptors (type 1 to type 5) and Opioid receptors (type D, K, M, X);

(c) receptors that bind to hormone proteins- Follic stimulating hormone receptor, Thyrotrophin receptor and Lutropin-choriogonadotropic hormone receptor;

(d) receptors that bind to neurotransmitters-substance P receptor, Substance K receptor and neuropeptide Y receptor;

(e) Olfactory receptors-Olfactory type 1 to type 11, Gustatory and odorant receptors;

(f) Prostanoid receptors-Prostaglandin E2 (EP1 to EP4 subtypes), Prostacyclin and Thromboxane;

(g) receptors that bind to metabotropic substances-Metabotropic glutamate group I to group III receptors;

(h) receptors that respond to physical stimuli, such as light, or to chemical stimuli, such as taste and smell; and

(i) orphan GPCRs-the natural ligand to the receptor is undefined.

ICAST™ provides many benefits to the screening process, including the ability to monitor protein interactions in any sub-cellular compartment-membrane, cytosol and nucleus; the ability to achieve a more physiologically relevant model without requiring protein overexpression; and the ability to achieve a functional assay for receptor binding allowing high information content.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. Cellular expression levels of β2 adrenergic receptor (β2AR) and β-arrestin-2 (βArr2) in C2 clones. Quantification of β-gal fusion protein was performed using antibodies against β-gal and purified β-gal protein in a titration curve by a standardized ELISA assay. Figure 1A shows expression levels of β2AR-βgalΔα clones (in expression vector pICAST ALC). Figure 1B shows expression levels of βArr2-βgalΔω in expression vector pICAST OMC4 for clones 9-3, -7, -9, -10, -19 and –24, or in expression vector pICAST OMN4 for clones 12-4, -9, -16, -18, -22 and -24.

FIGURE 2. Receptor β2AR activation was measured by agonist-stimulated cAMP production. C2 cells expressing pICAST ALC β2AR (clone 5) or parental cells were treated with increasing concentrations of (-)isoproterenol and 0.1mM IBMX. The quantification of cAMP level was expressed as pmol/well.

FIGURE 3. Interaction of activated receptor β2AR and arrestin can be measured by β-galactosidase complementation. Figure 3A shows a time course of β–galactosidase activity in response to agonist (-)isoproterenol stimulation in C2 expressing β2AR-βgalΔα (β2AR alone, in expression vector pICAST ALC), or C2 clones, and a pool of C2 co-expressing β2AR-βgalΔα and βArr2-βgalΔω (in expression vectors pICAST ALC and pICAST OMC). Figure 3B shows a time course of β galactosidase activity in response to agonist (-)isoproterenol stimulation in C2 cells expressing β2AR alone (in expression vector pICAST ALC) and C2 clones co-expressing β2AR and βArr1 (in expression vectors ICAST ALC and pICAST OMC).

FIGURE 4. Agonist dose response for interaction of β2AR and arrestin can be measured by β-galactosidase complementation. Figure 4A shows a dose response to agonists (-)isoproterenol and procaterol in C2 cells co-expressing pICAST ALC β2AR and pICAST OMC βArr2 fusion constructs. Figure 4B shows a dose response to agonists (-)isoproterenol and procaterol in C2 cells co-expressing pICAST ALC β2AR and pICAST OMC βArr1 fusion constructs.

FIGURE 5. Antagonist mediated inhibition of receptor activity can be measured by β-galactosidase complementation in cells co-expressing β2AR-βgalΔα and βArr-βgalΔω. Figure 5A shows specific inhibition with adrenergic antagonists ICI-118,551 and propranolol of β-galactosidase activity in C2 clones co-expressing pICAST ALC β2AR and pICAST OMC βArr2 fusion constructs after incubation with agonist (-)isoproterenol. Figure 5B shows specific inhibition of β-galactosidase activity with adrenergic antagonists ICI-118,551

and propranolol in C2 clones co-expressing pICAST ALC β2AR and pICAST OMC βArr1 fusion constructs in the presence of agonist (-)isoproterenol.

FIGURE 6. C2 cells expressing adenosine receptor A2a show cAMP induction in response to agonist (CGC-21680) treatment. C2 parental cells and C2 cells co-expressing pICAST ALC A2aR and pICAST OMC βArr1 as a pool or as selected clones were measured for agonist-induced cAMP response (pmol/well).

FIGURE 7. Agonist stimulated cAMP response in C2 cells co-expressing Dopamine receptor D1 (D1-βgalΔα) and β-arrestin-2 (βArr2-βgalΔω). The clone expressing βArr2-βgalΔω (Arr2 alone) was used as a negative control in the assay. Cells expressing D1-βgalΔα in addition to βArr2-βgalΔω responded agonist treatment (3-hydroxytyramine hydrochloride at 3 μM) . D1(PIC2) or D1(PIC3) designate D1 in expression vector pICAST ALC2 or pICAST ALC4, respectively.

FIGURE 8. Variety of mammalian cell lines can be used to generate stable cells for monitoring GPCR and arrestin interactions. FIGURE 8A, FIGURE 8B and FIGURE 8C show the examples of HEK293, CHO and CHW cell lines co-expressing adrenergic receptor β2AR and arrestin fusion proteins of β-galactosidase mutants. The β-galactosidase activity was used to monitor agonist-induced interaction of β2AR and arrestin proteins.

FIGURE 9. Beta-gal complementation can be used to monitor β2 adrenergic receptor homo-dimerization. FIGURE 9A shows β–galactosidase activity in HEK293 clones co-expressing pICAST ALC β2AR and pICAST OMC β2AR. FIGURE 9B shows a cAMP response to agonist (-)isoproterenol in HEK 293 clones co-expressing pICAST ALC β2AR

and pICAST OMC β2AR. HEK293 parental cells were included in the assays as negative controls.

FIGURE 10A. pICAST ALC: Vector for expression of β-galΔα as a C-terminal fusion to the target protein. This construct contains the following features: MCS, multiple cloning site for cloning the target protein in frame with the β-galΔα; GS Linker, (GGGGS)n; NeoR, neomycin resistance gene; IRES, internal ribosome entry site; ColE1ori, origin of replication for growth in E. coli; 5'MoMuLV LTR and 3'MoMuLV LTR, viral promotor and polyadenylation signals from the Moloney Murine leukemia virus.

FIGURE 10B. Nucleotide sequence for pICAST ALC.

FIGURE 11A. pICAST ALN: Vector for expression of β-galΔα as an N-terminal fusion to the target protein. This construct contains the following features: MCS, multiple cloning site for cloning the target protein in frame with the β-galΔα; GS Linker, (GGGGS)n; NeoR, neomycin resistance gene; IRES, internal ribosome entry site; ColE1ori, origin of replication for growth in E. coli; 5'MoMuLV LTR and 3'MoMuLV LTR, viral promotor and polyadenylation signals from the Moloney Murine leukemia virus.

FIGURE 11B. Nucleotide sequence for pICAST ALN.

FIGURE 12A. pICAST OMC: Vector for expression of β-galΔω as a C-terminal fusion to the target protein. This construct contains the following features: MCS, multiple cloning site for cloning the target protein in frame with the β-galΔω ; GS Linker, (GGGGS)n; Hygro, hygromycin resistance gene; IRES, internal ribosome entry site; ColE1ori, origin of replication for growth in E. coli; 5'MoMuLV LTR and 3'MoMuLV LTR, viral promotor and polyadenylation signals from the Moloney Murine leukemia virus.

FIGURE 12B. Nucleotide sequence for pICAST OMC.

FIGURE 13A. pICAST OMN: Vector for expression of β-galΔω as an N-terminal fusion to the target protein. This construct contains the following features: MCS, multiple cloning site for cloning the target protein in frame with the β-galΔω; GS Linker, (GGGGS)n; Hygro, hygromycin resistance gene; IRES, internal ribosome entry site; ColE1ori, origin of replication for growth in E. coli; 5'MoMuLV LTR and 3'MoMuLV LTR, viral promotor and polyadenylation signals from the Moloney Murine leukemia virus.

FIGURE 13B. Nucleotide sequence for pICAST OMN.

FIGURE 14. pICAST ALC βArr2: Vector for expression of β-galΔα as a C-terminal fusion to β-arrestin-2. The coding sequence of human β-arrestin-2 (Genebank Accession Number: NM_004313) was cloned in frame to β-galΔα in a pICAST ALC vector.

FIGURE 15. pICAST OMC βArr2: Vector for expression of β-galΔω as a C-terminal fusion to β-arrestin-2. The coding sequence of human β-arrestin-2 (Genebank Accession Number: NM_004313) was cloned in frame to β-galΔω in a pICAST OMC vector.

FIGURE 16. pICAST ALC βArr1: Vector for expression of β-galΔα as a C-terminal fusion to β-arrestin-1. The coding sequence of human β-arrestin-1 (Genebank Accession Number: NM_004041) was cloned in frame to β-galΔα in a pICAST ALC vector.

FIGURE 17. pICAST OMC βArr1: Vector for expression of β-galΔω as a C-terminal fusion to β-arrestin-1. The coding sequence of human β-arrestin-1 (Genebank Accession Number: NM_004041) was cloned in frame to β-galΔω in a pICAST OMC vector.

FIGURE 18. pICAST ALC β2AR: Vector for expression of β-galΔα as a C-terminal fusion to β2 Adrenergic Receptor. The coding sequence of human β2 Adrenergic Receptor

(Genebank Accession Number: NM_000024) was cloned in frame to β-galΔα in a pICAST ALC vector.

FIGURE 19. pICAST OMC β2AR: Vector for expression of β-galΔω as a C-terminal fusion β2 Adrenergic Receptor. The coding sequence of human β2 Adrenergic Receptor (Genebank Accession Number: NM_000024) was cloned in frame to β-galΔω in a pICAST OMC vector.

FIGURE 20. pICAST ALC A2aR: Vector for expression of β-galΔα as a C-terminal fusion to Adenosine 2a Receptor. The coding sequence of human Adenosine 2a Receptor (Genebank Accession Number: NM_000675) was cloned in frame to β-galΔα in a pICAST ALC vector.

FIGURE 21. pICAST OMC A2aR: Vector for expression of β-galΔω as a C-terminal fusion to Adenosine 2a Receptor. The coding sequence of human Adenosine 2a Receptor (Genebank Accession Number: NM_000675) was cloned in frame to β-galΔω in a pICAST OMC vector.

FIGURE 22. pICAST ALC D1: Vector for expression of β-galΔα as a C-terminal fusion to Dopamine D1 Receptor. The coding sequence of human Dopamine D1 Receptor (Genebank Accession Number: X58987) was cloned in frame to β-galΔα in a pICAST ALC vector.

FIGURE 23. A schematic depicting the method of the invention, which shows that two inactive mutants that become active when they interact.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

All literature and patents cited in this disclosure are incorporated herein by reference.

The present invention provides a method to interrogate GPCR function and pathways. The G-protein-coupled superfamily continues to expand rapidly as new receptors are discovered through automated sequencing of cDNA libraries or genomic DNA. It is estimated that several thousand GPCRs may exist in the human genome, as many as 250 GPCRs have been cloned and only as few as 150 have been associated with ligands. The means by which these, or newly discovered orphan receptors, will be associated with their cognate ligands and physiological functions represents a major challenge to biological and biomedical research. The identification of an orphan receptor generally requires an individualized assay and a guess as to its function. The interrogation of a GPCR's signaling behavior by introducing a replacement receptor eliminates these prerequisites because it can be performed with and without prior knowledge of other signaling events. It is sensitive, rapid and easily performed and should be applicable to nearly all GPCRs because the majority of these receptors should desensitize by a common mechanism.

Various approaches have been used to monitor intracellular activity in response to a stimulant, e.g., enzyme-linked immunosorbent assay (ELISA); Fluorescense Imaging Plate Reader assay (FLIPR™, Molecular Devices Corp., Sunnyvale, CA); EVOscreen™, EVOTEC™, Evotec Biosystems Gmbh, Hamburg, Germany; and techniques developed by CELLOMICS™, Cellomics, Inc., Pittsburgh, PA.

Germino, F.J., et al., "Screening for in vivo protein-protein interactions." Proc. Natl. Acad. Sci., 90(3): 933-7 (1993), discloses an *in vivo* approach for the isolation of proteins interacting with a protein of interest.

Phizicky, E.M., et al., "Protein-protein interactions: methods for detection and analysis." Microbiol. Rev., 59(1): 94-123 (1995), discloses a review of biochemical, molecular biological and genetic methods used to study protein-protein interactions.

Offermanns, et al., "$G\alpha_{15}$ and $G\alpha_{16}$ Couple a Wide Variety of Receptors to Phospholipase C." J. Biol. Chem., 270(25):15175-80 (1995), discloses that $G\alpha_{15}$ and $G\alpha_{16}$ can be activated by a wide variety of G-protein-coupled receptors. The selective coupling of an activated receptor to a distinct pattern of G-proteins is regarded as an important requirement to achieve accurate signal transduction. Id.

Barak et al., "A β-arrestin/Green Fluorescent Protein Biosensor for Detecting G Protein-coupled Receptor Activation." J. Biol. Chem., 272(44):27497-500 (1997) and U.S. Patent No. 5,891,646, disclose the use of a β-arrestin/green fluorescent fusion protein (GFP) to monitor protein translocation upon stimulation of GPCR.

The present invention involves a method for monitoring protein-protein interactions in GPCR pathways as a complete assay using ICAST™ (Intercistronic Complementation Analysis Screening Technology as disclosed in pending U.S. patent application serial no. 053,164, filed April 1, 1998, the entire contents of which are incorporated herein by reference). This invention enables an array of assays, including GPCR binding assays, to be achieved directly within the cellular environment in a rapid, non-radioactive assay format amenable to high-throughput screening. Using existing technology, assays of this type are currently performed in a non-cellular environment and require the use of radioisotopes.

The present invention combined with Tropix ICAST™ and Advanced Discovery Sciences™ technologies, e.g., ultra high-throughput screening, provide highly sensitive cell-based methods for interrogating GPCR pathways which are amendable to high-throughput

screening (HTS). These methods are an advancement over the invention disclosed in U.S. Patent 5,891,646, which relies on microscopic imaging of GPCR components as fusion with Green-fluorescent-protein. Imaging techniques are limited by low-throughput, lack of thorough quantification and low signal to noise ratios. Unlike yeast-based-2-hybrid assays used to monitor protein/protein interactions in high-throughput assays, the present invention is applicable to a variety of cells including mammalian cells, plant cells, protozoa cells such as E. coli and cells of invertebrate origin such as yeast, slime mold (*Dictyostelium*) and insects; detects interactions at the site of the receptor target or downstream target proteins rather than in the nucleus; and does not rely on indirect read-outs such as transcriptional activation. The present invention provides assays with greater physiological relevance and fewer false negatives.

Advanced Discovery Sciences™ is in the business of offering custom-developed screening assays optimized for individual assay requirements and validated for automation. These assays are designed by HTS experts to deliver superior assay performance. Advanced Discovery Sciences'™ custom assay development service encompasses the design, development, optimization and transfer of high performance screening assays. Advanced Discovery Sciences™ works to design new assays or convert existing assays to ultra-sensitive luminescent assays ready for the rigors of HTS. Among some of the technologies developed by Advanced Discovery Sciences™ are the cAMP-Screen™ immunoassay system. This system provides ultrasensitive determination of cAMP levels in cell lysates. The cAMP-Screen™ assay utilizes the high-sensitivity chemiluminescent alkaline phosphatase (AP) substrate CSPD® with Sapphire-II™ luminescence enhancer.

EXAMPLE:

GPCR activation can be measured through monitoring the binding of ligand-activated GPCR by an arrestin. In this assay system, a GPCR, e.g. β adrenergic receptor (β 2AR) and a β arrestin are co-expressed in the same cell as fusion proteins with β gal mutants. As illustrated in Figure 1, the β2AR is expressed as a fusion protein with Δα form of β gal mutant (β2ADRΔα) and the b arrestin as a fusion protein with the Δω mutant of β gal (β-ArrΔω). The two fusion proteins exist inside of a resting (or un-stimulated) cell in separate compartments, i.e. membrane for GPCR and cytosol for arrestin, and they can not form an active b galactosidase enzyme. When such a cell is treated with an agonist or a ligand, the ligand-occupied and activated receptor will become a high affinity binding site for Arrestin. The interaction between an activated β2ADRΔα and β–ArrΔω drives the β gal gal mutant complementation. The enzyme activity can be measured by using an enzyme substrate, which upon cleavage releases a product measurable by colorimetry, fluorescence, chemiluminescence (e.g. Tropix product GalScreenTM).

Experiment protocol-

1. In the first step, the expression vectors for β2ADRΔα and βArr2Δω were engineered in selectable retroviral vectors pICAST ALC, as described in Figure 18 and pICAST OMC, as in Figure 15.

2. In the second step, the two expression constructs were transduced into either C2C12 myoblast cells, or other mammalian cell lines, such as COS-7, CHO, A431, HEK 293, and CHW. Following selection with antibiotic drugs, stable clones expressing both fusion

proteins at appropriate levels were selected.

3. In the last step, the cells expressing both β2ADRΔα and βArr2Δω were tested for response by agonist/ligand stimulated β galactosidase activity. Triplicate samples of cells were plated at 10,000 cells in 100 microliter volume into a well of 96-well culture plate. Cells were cultured for 24 hours before assay. For agonist assay (Figure 3 and 4), cells were treated with variable concentrations of agonist, for example, (-) isoproterenol, procaterol, dobutamine, terbutiline or L-L-phenylephrine for 60 min at 37 C. The induced β galatosidase activity was measured by addition of Tropix GalScreenTM substrate (Applied Biosystems) and luminescence measured in a Tropix TR717TM luminometer (Applied Biosystems). For antagonist assay (Figure 5), cells were pre-incubated for 10 min in fresh medium without serum in the presence of ICI-118,551 or propranolol followed by addition of 10 micro molar (-) isoproterenol.

The assays of this invention, and their application and preparation have been described both generically, and by specific example. The examples are not intended as limiting. Other substituent identities, characteristics and assays will occur to those of ordinary skill in the art, without the exercise of inventive faculty. Such modifications remain within the scope of the invention, unless excluded by the express recitation of the claims advanced below.

WHAT IS CLAIMED IS:

1. A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to one mutant form of reporter enzyme and an interacting protein partner as a fusion to another mutant form of enzyme;

b) exposing the cell to a ligand for said GPCR under said test condition; and

c) monitoring activation of said GPCR by complementation of said reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of said test condition indicates increased GPCR interaction with its interacting protein partner compared to that which occurs in the absence of said test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of said test condition indicates decreased GPCR interaction with its interacting protein partner compared to that which occurs in the absence of said test condition.

2. A method according to Claim 1, wherein the test condition is the presence in the cell of a kinase.

3. A method according to Claim 1, wherein the test condition is the presence in the cell of a G-protein.

4. A method according to Claim 1, wherein the test condition is the exposure of the cell to a compound selected from GPCR agonists and GPCR antagonists.

5. A method according to Claim 1, wherein the test condition is co-expression in the cell of a second receptor.

6. A method according to Claim 5, wherein the second receptor is a GPCR receptor.

7. A method according to Claim 5, wherein homo-dimerization of GPCR is determined.

8. A method according to Claim 5, wherein hetero-dimerization of GPCR is determined.

9. A method for screening a β-arrestin protein or an unidentified arrestin or arrestin-like protein or fragment and mutant form thereof for the ability to bind to activated GPCRs, comprising:

a) providing a cell that:

i) expresses at least one GPCR as a fusion protein to a reporter enzyme; and

ii) contains a conjugate comprising a test β-arrestin protein as a fusion protein with another reporter enzyme;

b) exposing the cell to a ligand for said at least one GPCR; and

c) detecting enzymatic activity of the complemented reporter enzyme;

wherein an increase in enzymatic activity in the cell indicates β-arrestin protein binding to the activated GPCR.

10. A method for screening a test compound for G-protein-coupled receptor (GPCR) agonist activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to one mutant form of reporter enzyme and an arrestin protein as a fusion to another mutant form of enzyme;

b) exposing the cell to a test compound; and

c) detecting complementation of said reporter enzyme;

wherein increased reporter enzyme activity after exposure of the cell to the test compound indicates GPCR agonist activity of the test compound.

11. A method according to Claim 10, wherein the cell expresses a GPCR whose function is known.

12. A method according to Claim 10, wherein the cell expresses a GPCR whose function is unknown.

13. A method according to Claim 10, wherein the cell expresses an odorant or taste GPCR.

14. A method according to Claim 10, wherein the cell expresses a GPCR a β-adrenergic GPCR.

15. A method according to Claim 10, wherein the cell is selected from the group consisting of mammalian cells, cells of invertebrate origin, plant cells and protozoa cells.

16. A method according to Claim 10, wherein the cell endogenously expresses a GPCR.

17. A method according to Claim 10, wherein the cell has been transformed to express a GPCR not endogenously expressed by such a cell.

18. A method of screening a test compound for G-protein-coupled receptor (GPCR) antagonist activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to one mutant form of reporter enzyme and an arrestin protein as a fusion to another mutant form of enzyme;

b) exposing the cell to said test compound;

c) exposing the cell to an agonist for said GPCR; and

d) detecting complementation of said reporter enzyme;

where exposure to the agonist occurs at the same time as, or subsequent to, exposure to the test compound, and wherein decreased reporter enzyme activity after exposure of the

cell to the test compound indicates that the test compound is an antagonist for said GPCR.

19. A method of screening a cell for the presence of a G-protein-coupled receptor (GPCR) responsive to a GPCR agonist, comprising:

a) providing a cell, said cell containing a conjugate comprising a β-arrestin protein as a fusion protein with a reporter enzyme;

b) exposing the cell to a GPCR agonist; and

c) detecting enzymatic activity of the reporter enzyme;

wherein an increase in enzymatic activity after exposure of the cell to the GPCR agonist indicates that the cell contains a GPCR responsive to said agonist.

20. A method of screening a plurality of cells for those cells which contain a G-protein-coupled receptor (GPCR) responsive to a GPCR agonist, comprising:

a) providing a plurality of cells, said cells containing a conjugate comprising a β-arrestin protein as a fusion protein with a reporter enzyme;

b) exposing the cells to a GPCR agonist; and

c) detecting enzymatic activity of the reporter enzyme;

wherein an increase in enzymatic activity after exposure to the GPCR agonist indicates β-arrestin protein binding to a GPCR, thereby indicating that the cell contains a GPCR responsive to said GPCR agonist.

21. A method according to Claim 20, wherein the plurality of cells are contained in a tissue.

22. A method according to Claim 20, wherein the plurality of cells are contained in an organ.

23. A method according to Claim 20, wherein step (b) comprises exposing the cells to a plurality of GPCR agonists or ligand libraries.

24. A substrate having deposited thereon a plurality of cells, said cells expressing at least one GPCR as a fusion protein to one mutant form of reporter enzyme and an arrestin protein as a fusion to another mutant form of enzyme.

25. A substrate according to Claim 24, wherein the substrate contains an enzyme-labile chemical group which, upon cleavage by the reporter enzyme, releases a product measurable by colorimetry, fluorescence or chemiluminescence.

26. A substrate according to Claim 24, wherein the substrate is made of a material selected from glass, plastic, ceramic, semiconductor, silica, fiber optic, diamond, biocompatible monomer and biocompatible polymer materials.

27. A method of detecting G-protein-coupled receptor (GPCR) pathway activity in a cell expressing at least one GPCR and containing β-arrestin protein as a fusion protein with a reporter enzyme; wherein said enzymatic activity indicates activation of the GPCR pathway.

28. A method according to Claim 27, where the cells are deposited on a substrate prior to detecting said enzymatic activity.

29. A method according to Claim 27, wherein said cell is contained in a tissue.

30. A method according to Claim 27, wherein said cell is contained in an organ.

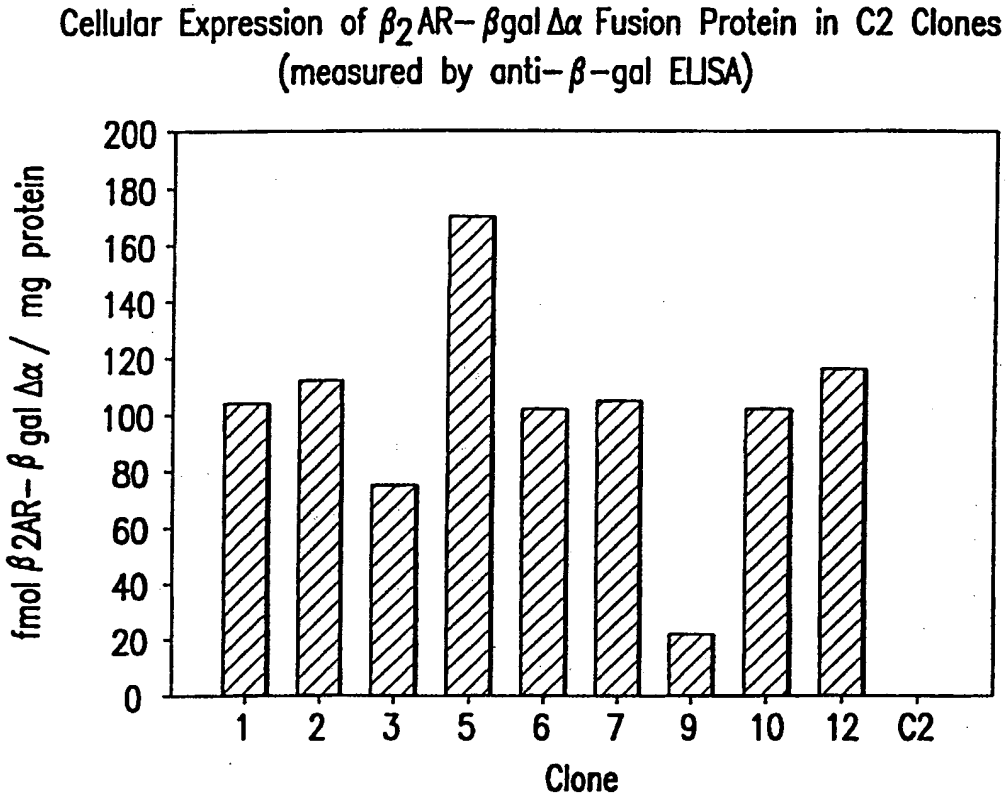
Cellular Expression of β_2AR–βgal$\Delta\alpha$ Fusion Protein in C2 Clones
(measured by anti–β–gal ELISA)
fmol β2AR–βgal$\Delta\alpha$ / mg protein
200
180
160
140
120
100
80
60
40
20
0
1
2
3
5
6
7
9
10
12
C2
Clone

FIG. 1A

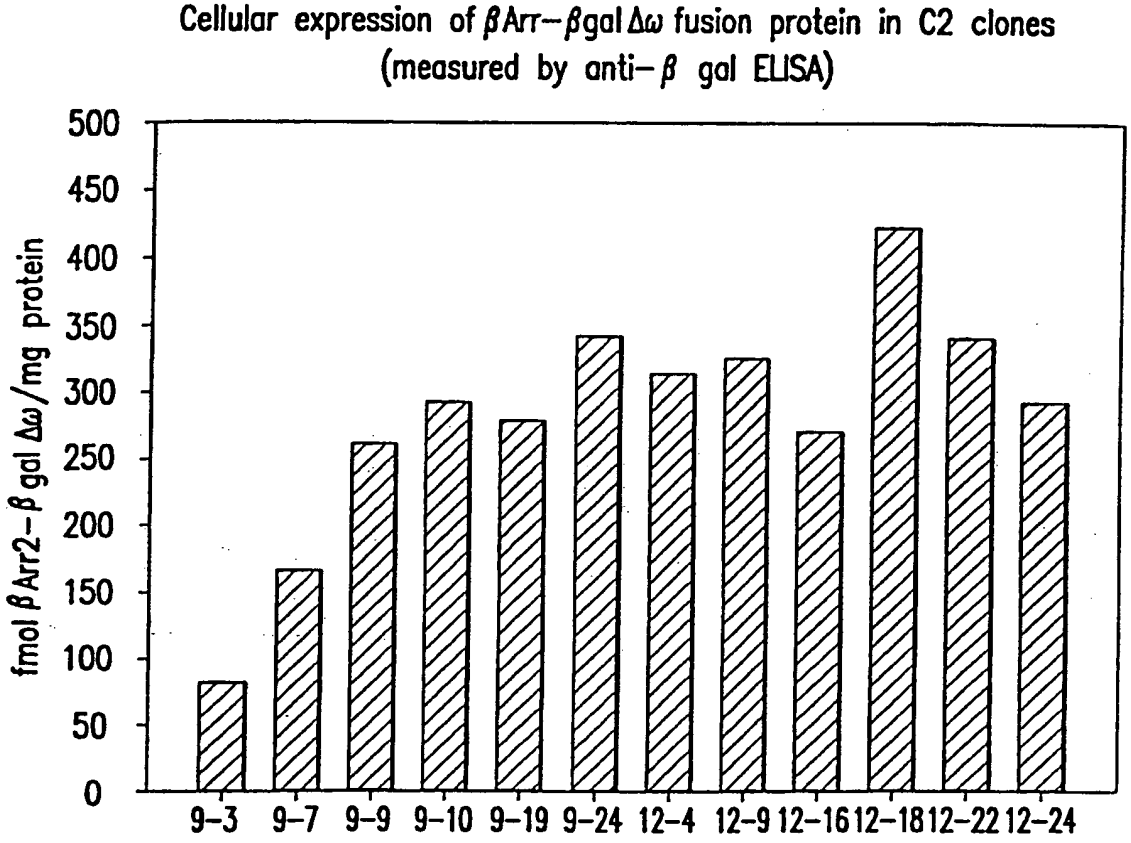
Cellular expression of βArr–βgal Δω fusion protein in C2 clones
(measured by anti–β gal ELISA)
fmol βArr2–β gal Δω/mg protein
500
450
400
350
300
250
200
150
100
50
0
9–3
9–7
9–9
9–10
9–19
9–24
12–4
12–9
12–16
12–18
12–22
12–24

FIG. 1B

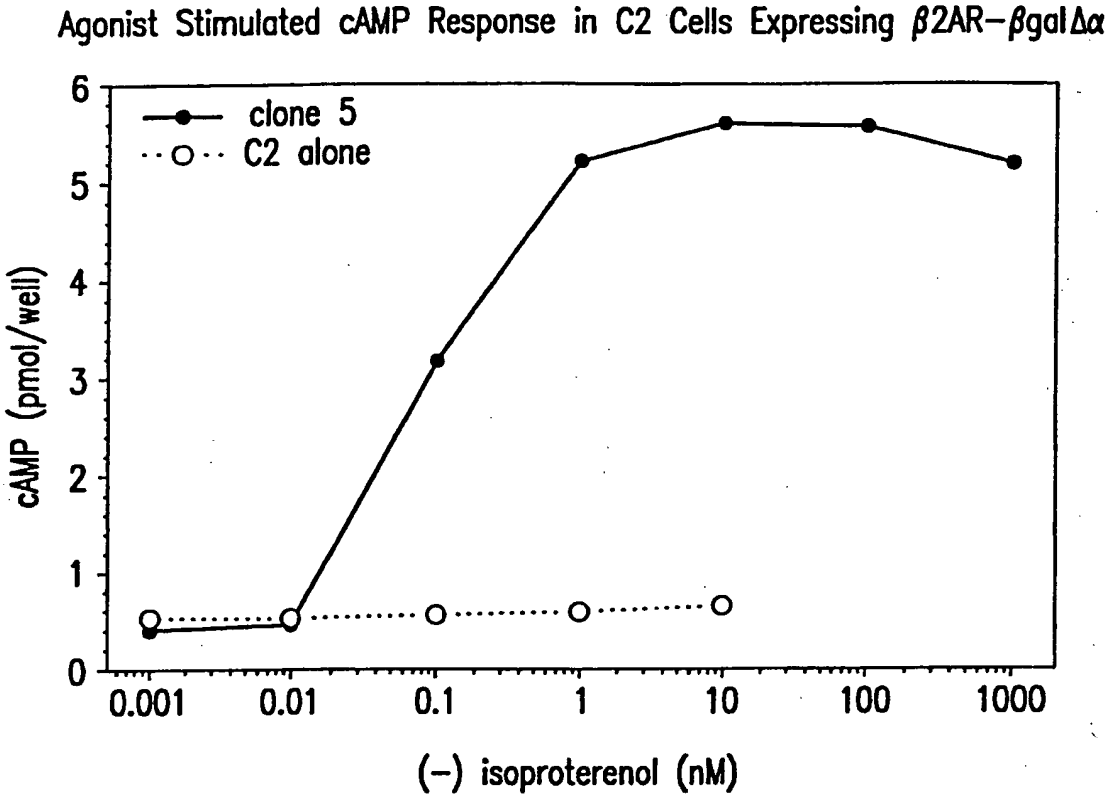
Agonist Stimulated cAMP Response in C2 Cells Expressing β2AR–βgalΔα
clone 5
C2 alone
cAMP (pmol/well)
0
1
2
3
4
5
6
0.001
0.01
0.1
1
10
100
1000
(–) isoproterenol (nM)

FIG.2

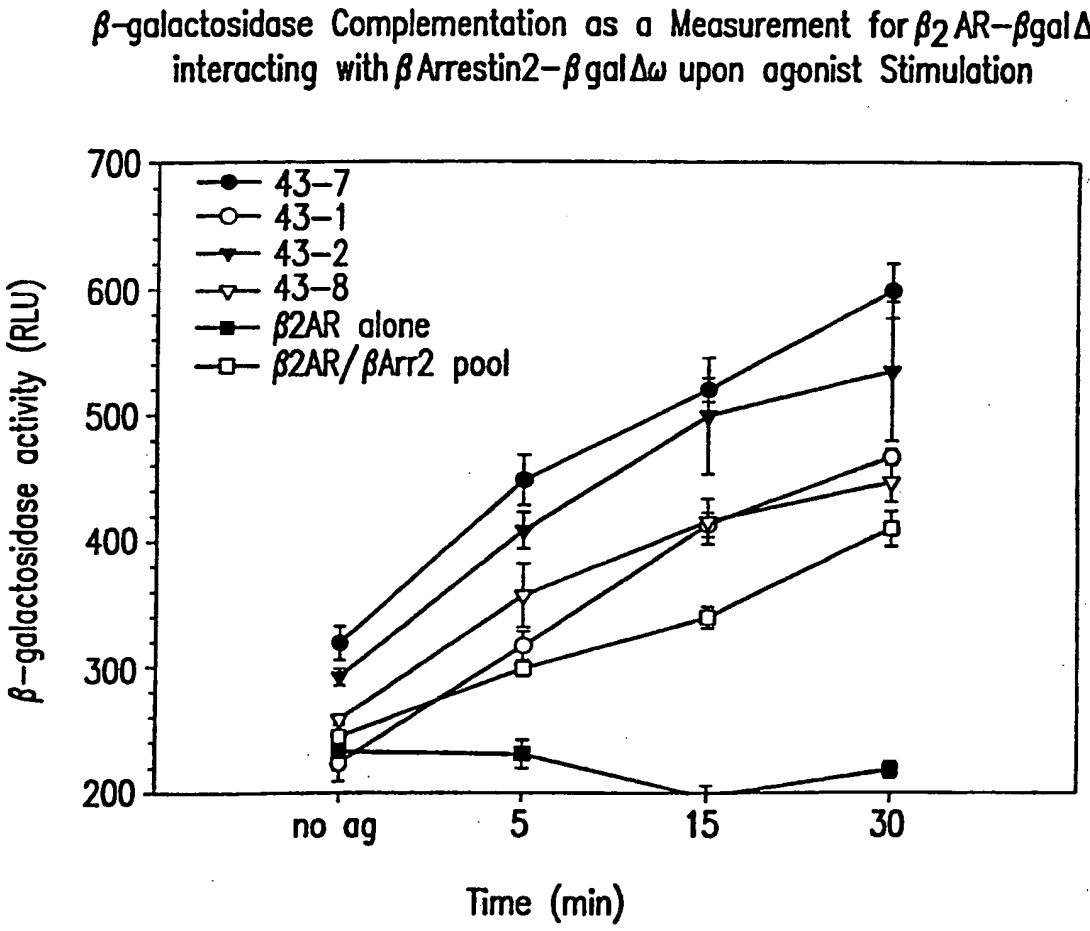
β-galactosidase Complementation as a Measurement for β2 AR–βgal Δα interacting with β Arrestin2–β gal Δω upon agonist Stimulation
43–7
43–1
43–2
43–8
β2AR alone
β2AR/βArr2 pool
β-galactosidase activity (RLU)
700
600
500
400
300
200
no ag
5
15
30
Time (min)

FIG. 3A

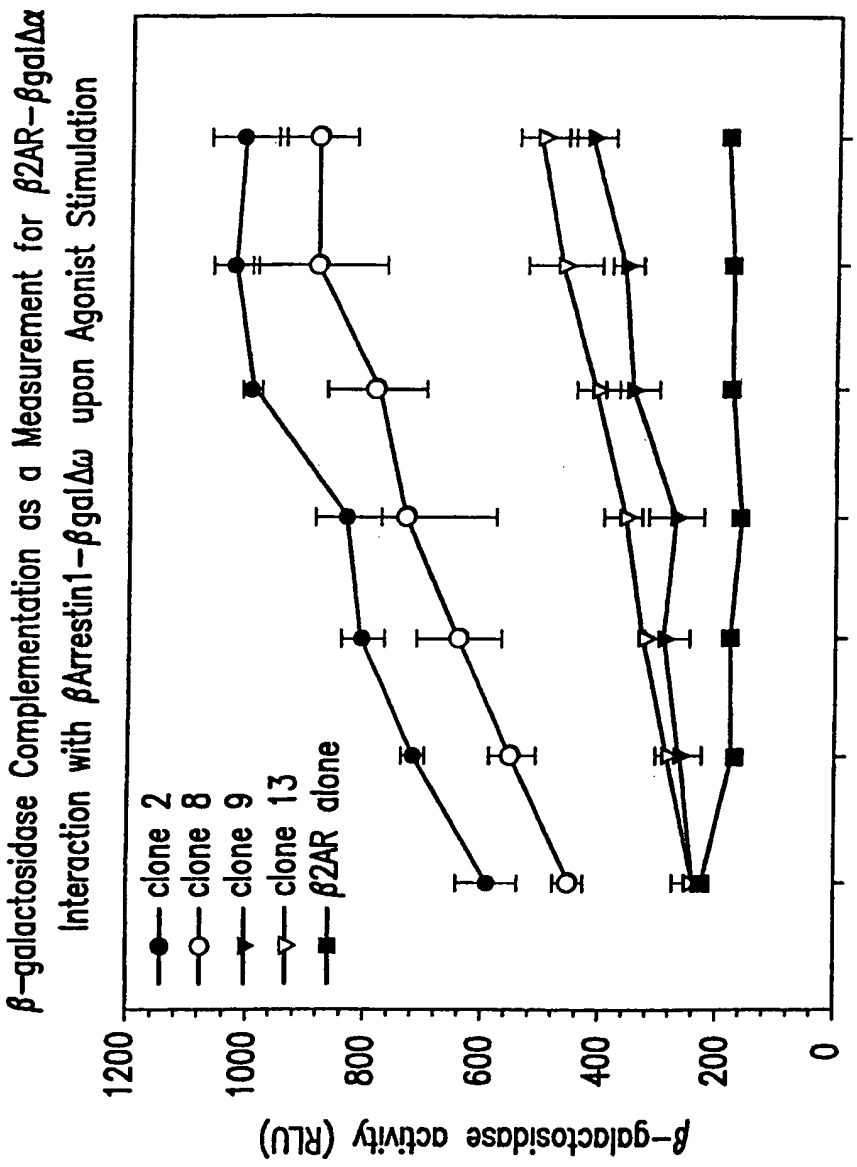
β-galactosidase Complementation as a Measurement for β2AR-βgalΔα
Interaction with βArrestin1-βgalΔω upon Agonist Stimulation
clone 2
clone 8
clone 9
clone 13
β2AR alone
β-galactosidase activity (RLU)
0
200
400
600
800
1000
1200
no ag
1
5
15
30
45
60
TIME (min)

FIG. 3B

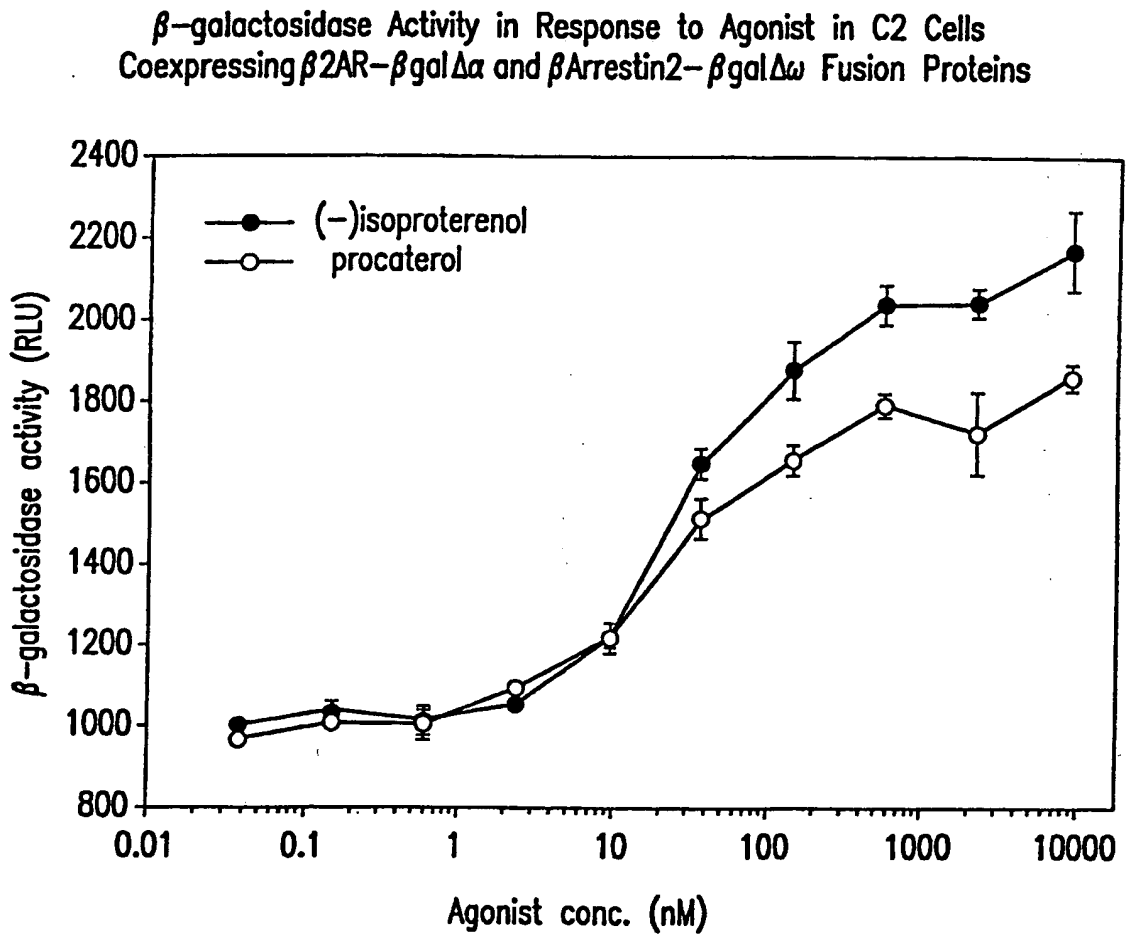
β-galactosidase Activity in Response to Agonist in C2 Cells
Coexpressing β2AR-βgalΔα and βArrestin2-βgalΔω Fusion Proteins
(-)isoproterenol
procaterol
β-galactosidase activity (RLU)
2400
2200
2000
1800
1600
1400
1200
1000
800
0.01
0.1
1
10
100
1000
10000
Agonist conc. (nM)

FIG. 4A

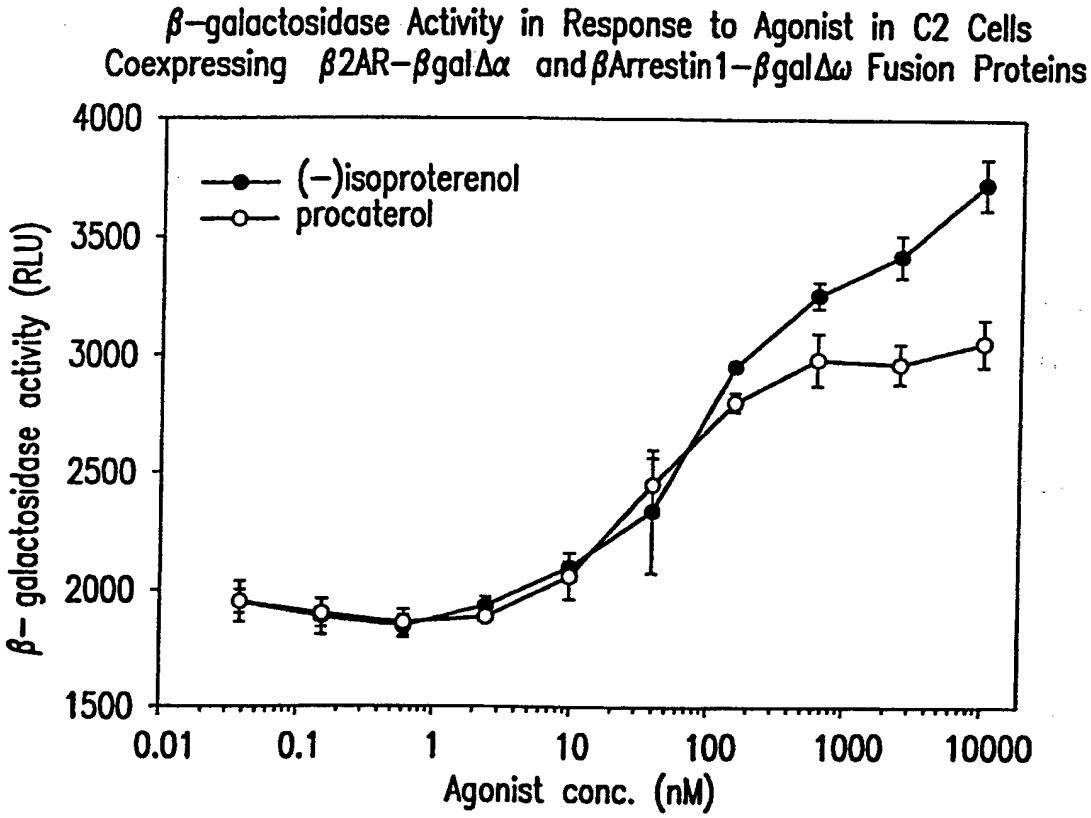
β–galactosidase Activity in Response to Agonist in C2 Cells
Coexpressing β2AR–βgalΔα and βArrestin1–βgalΔω Fusion Proteins
(–)isoproterenol
procaterol
β– galactosidase activity (RLU)
4000
3500
3000
2500
2000
1500
0.01
0.1
1
10
100
1000
10000
Agonist conc. (nM)

FIG. 4B

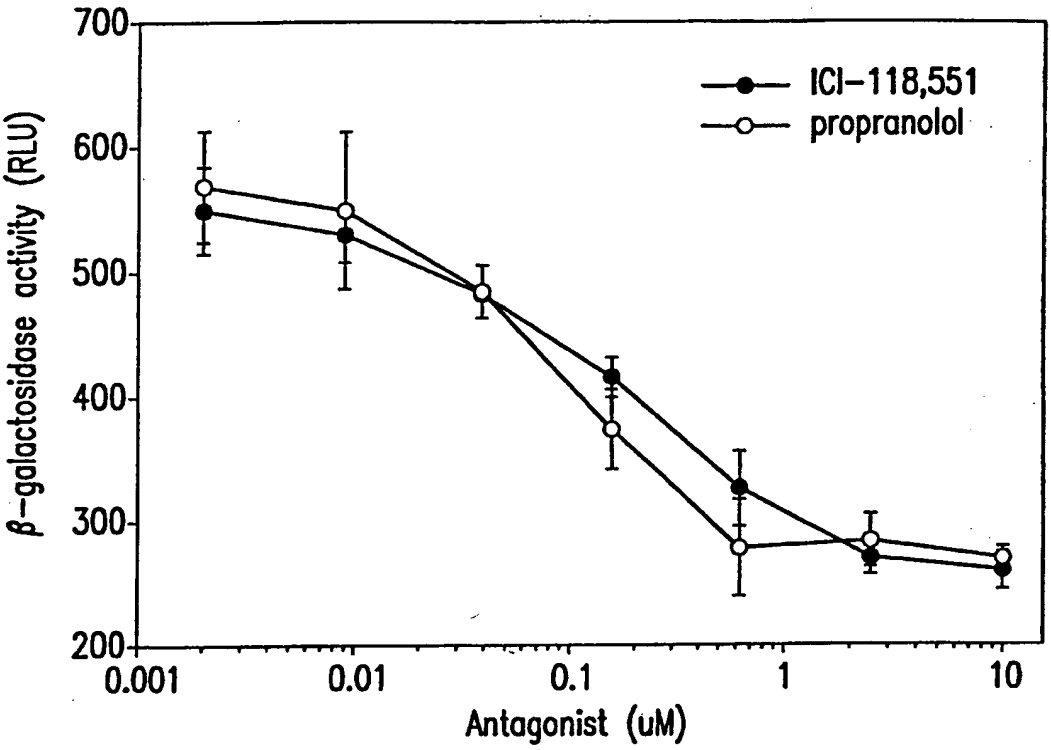
Inhibition of β-galactosidase activity in C2 Cells Coexpressing β2AR -βgal Δα and βArrestin2- βgal Δω Fusion Proteins
β-galactosidase activity (RLU)
700
600
500
400
300
200
0.001
0.01
0.1
1
10
Antagonist (uM)
ICI-118,551
propranolol

FIG. 5A

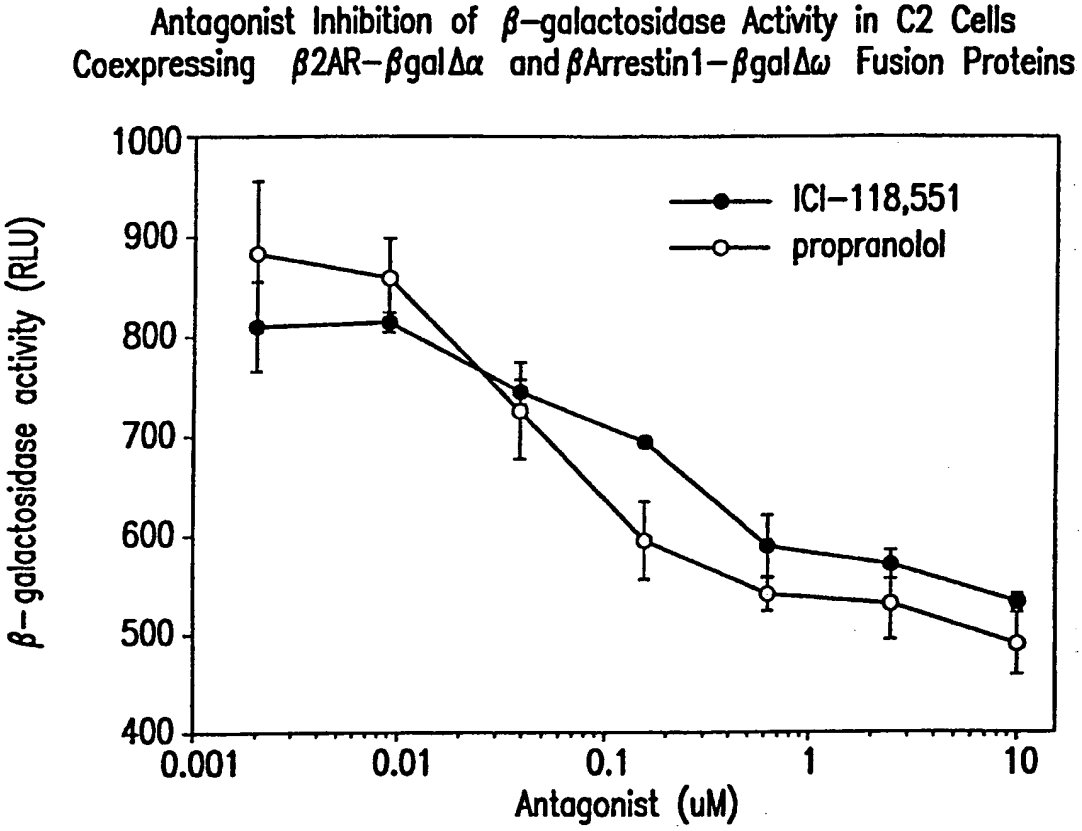
Antagonist Inhibition of β-galactosidase Activity in C2 Cells
Coexpressing β2AR-βgalΔα and βArrestin1-βgalΔω Fusion Proteins
β-galactosidase activity (RLU)
1000
900
800
700
600
500
400
0.001
0.01
0.1
1
10
Antagonist (uM)
ICI-118,551
propranolol

FIG. 5B

Agonist Stimulated cAMP Response in Clones or Pools of C2 Cells Coexpressing A2aR–βgal Δα and βArrestin1– βgal Δω Fusion Proteins

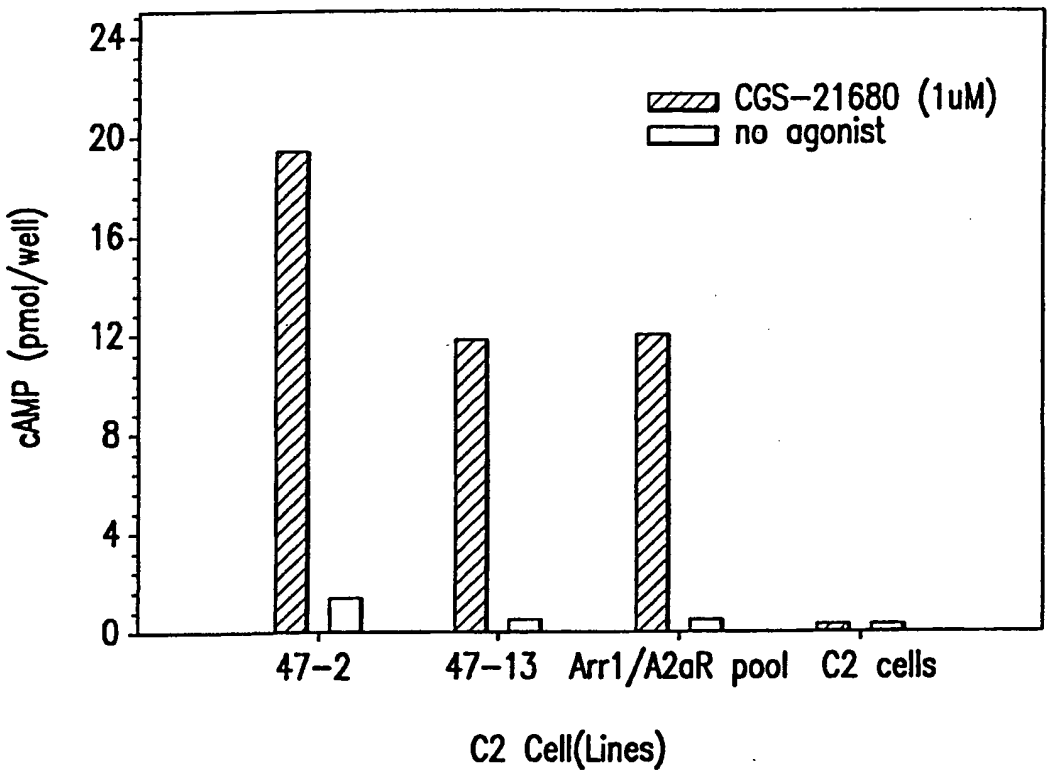

FIG.6

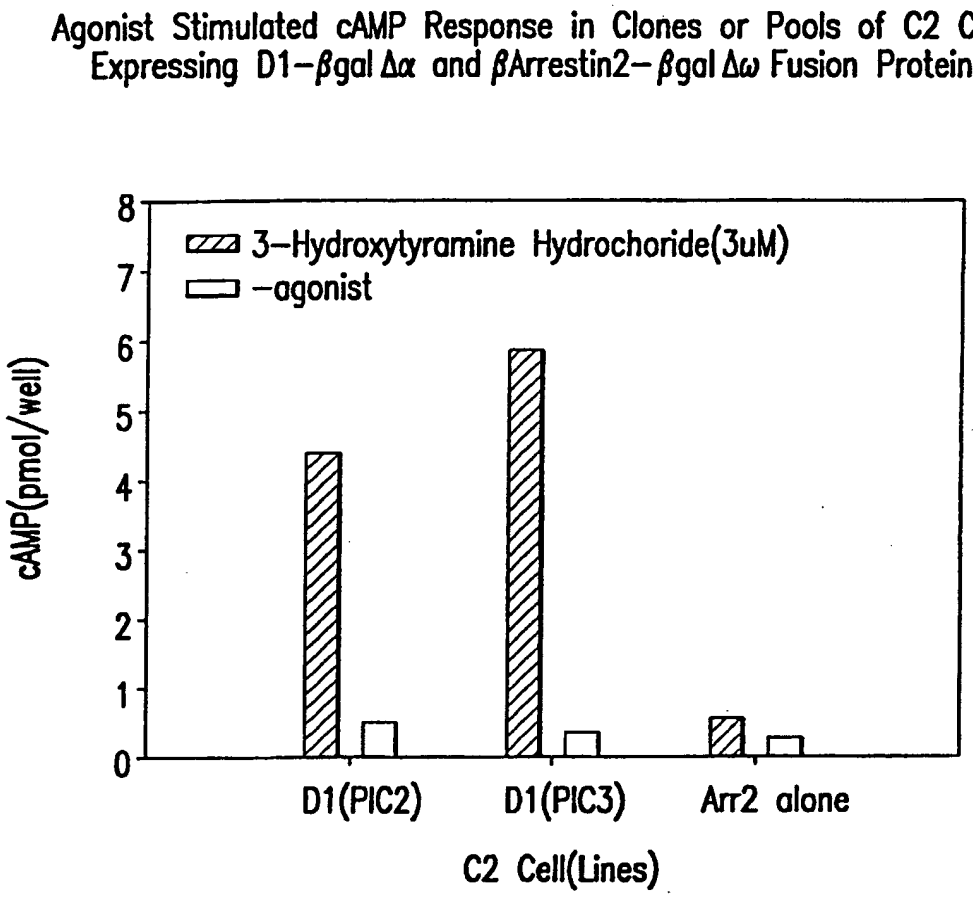
Agonist Stimulated cAMP Response in Clones or Pools of C2 Cells Expressing D1–βgal Δα and βArrestin2–βgal Δω Fusion Proteins
3–Hydroxytyramine Hydrochoride(3uM)
–agonist
cAMP(pmol/well)
0
1
2
3
4
5
6
7
8
D1(PIC2)
D1(PIC3)
Arr2 alone
C2 Cell(Lines)

FIG. 7

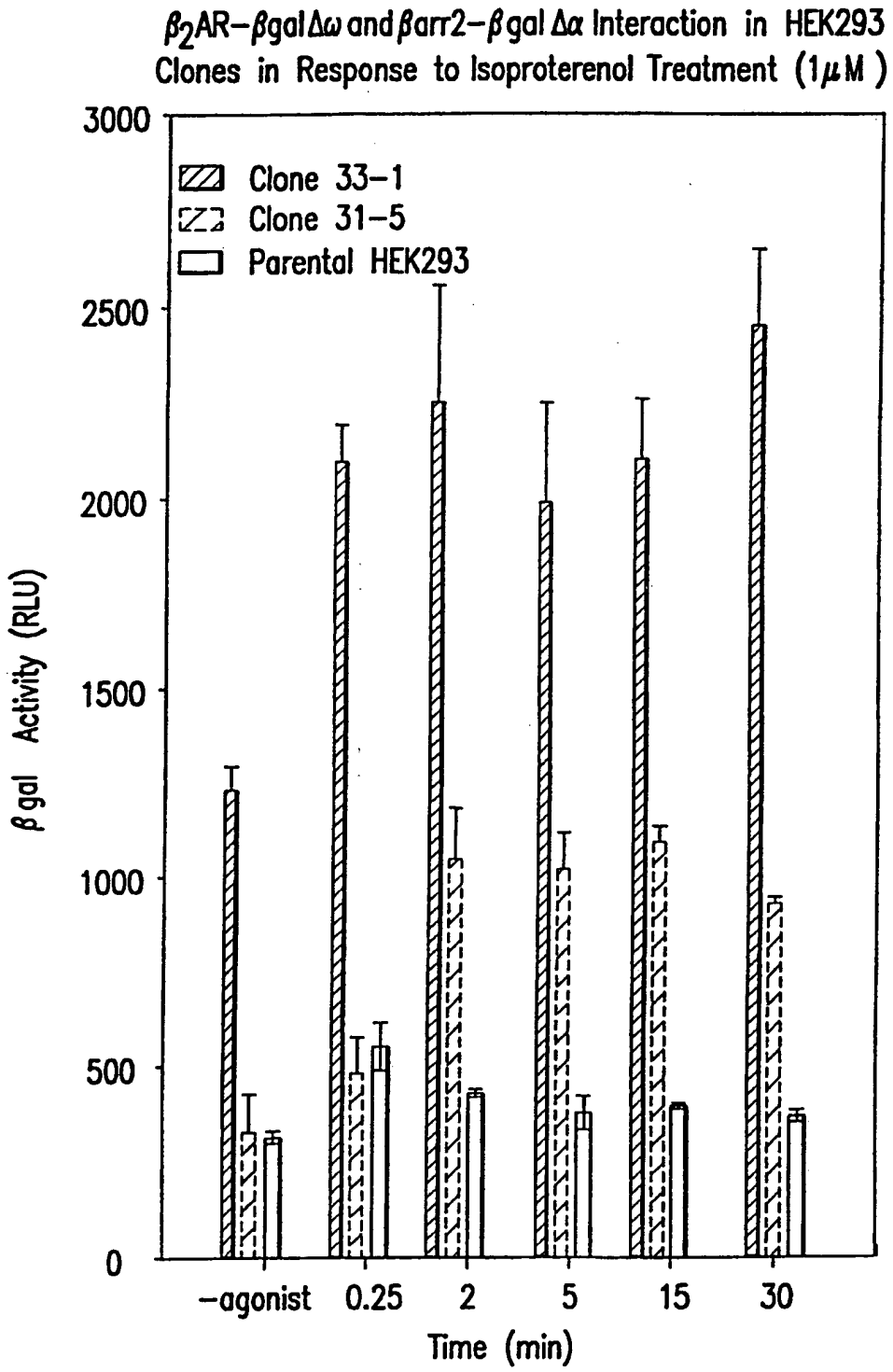
β2AR−βgalΔω and βarr2−βgal Δα Interaction in HEK293
Clones in Response to Isoproterenol Treatment (1μM)
Clone 33−1
Clone 31−5
Parental HEK293
βgal Activity (RLU)
3000
2500
2000
1500
1000
500
0
−agonist
0.25
2
5
15
30
Time (min)

FIG. 8A

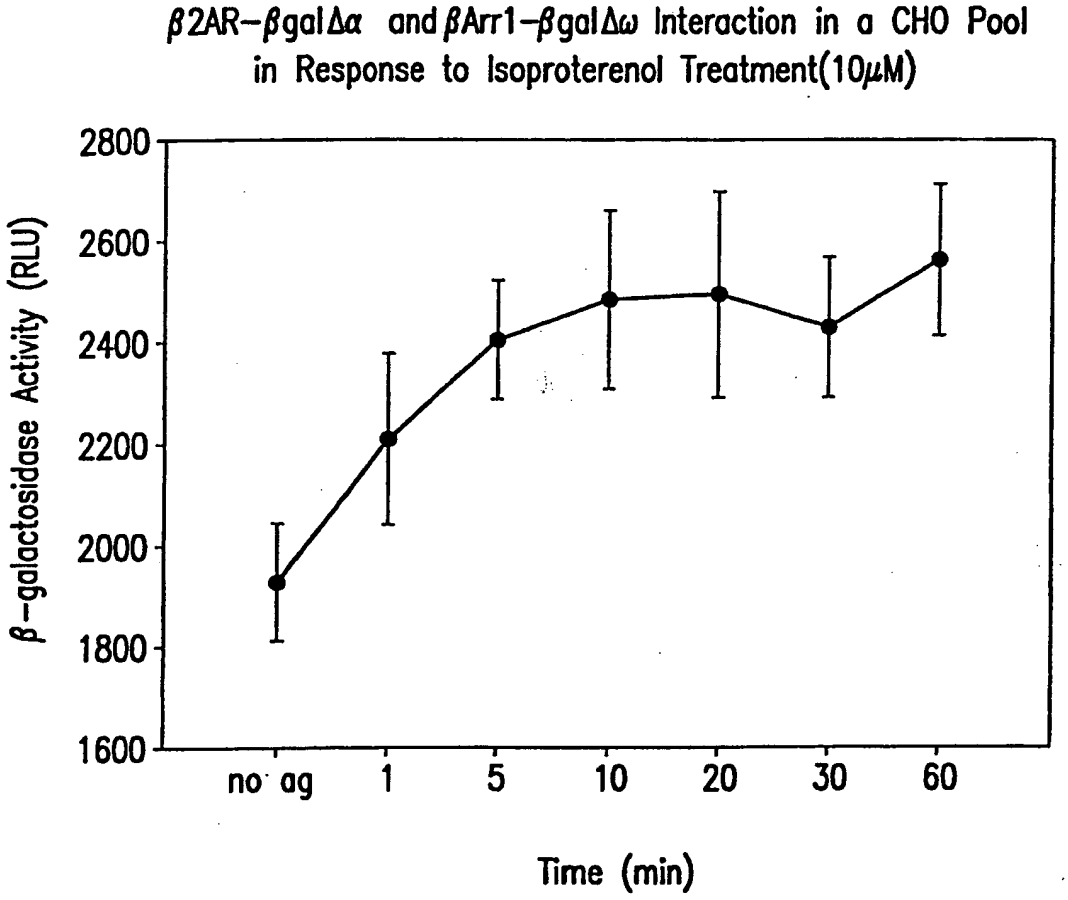
β2AR–βgalΔα and βArr1–βgalΔω Interaction in a CHO Pool
in Response to Isoproterenol Treatment(10μM)
β–galactosidase Activity (RLU)
2800
2600
2400
2200
2000
1800
1600
no ag
1
5
10
20
30
60
Time (min)

FIG. 8B

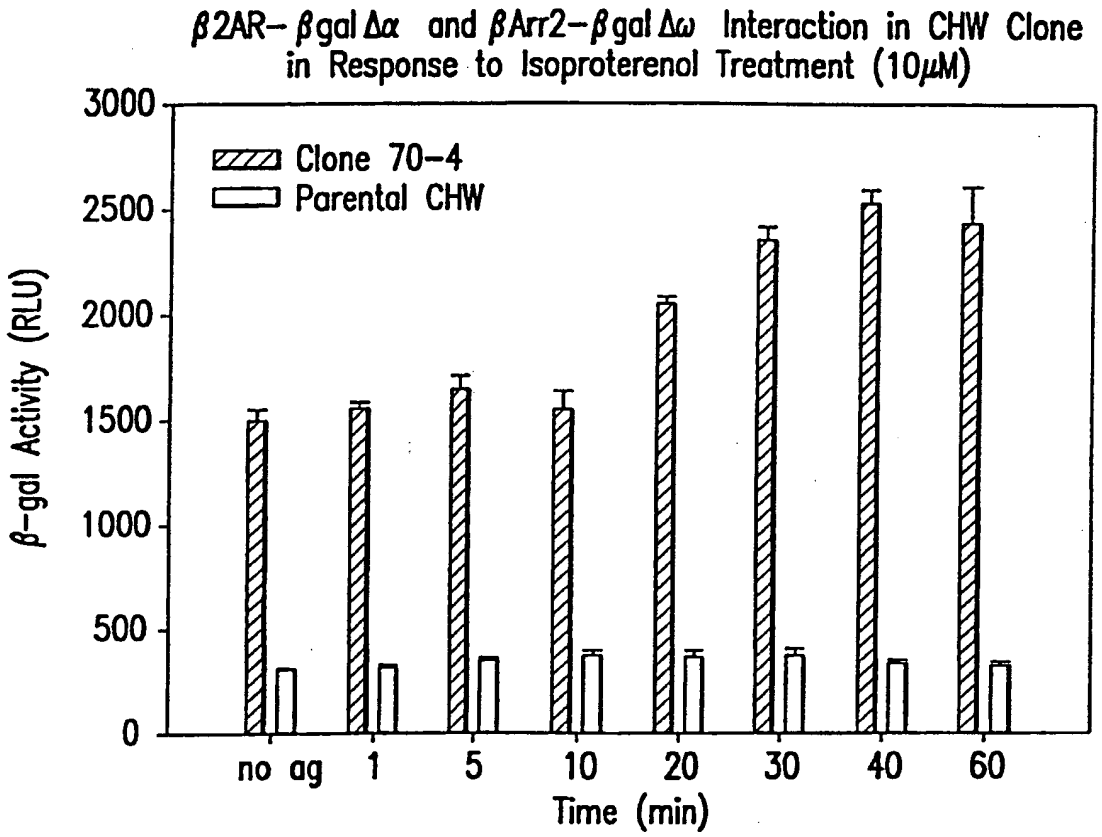
β2AR–βgal Δα and βArr2–βgal Δω Interaction in CHW Clone
in Response to Isoproterenol Treatment (10μM)
Clone 70–4
Parental CHW
β-gal Activity (RLU)
3000
2500
2000
1500
1000
500
0
no ag
1
5
10
20
30
40
60
Time (min)

FIG. 8C

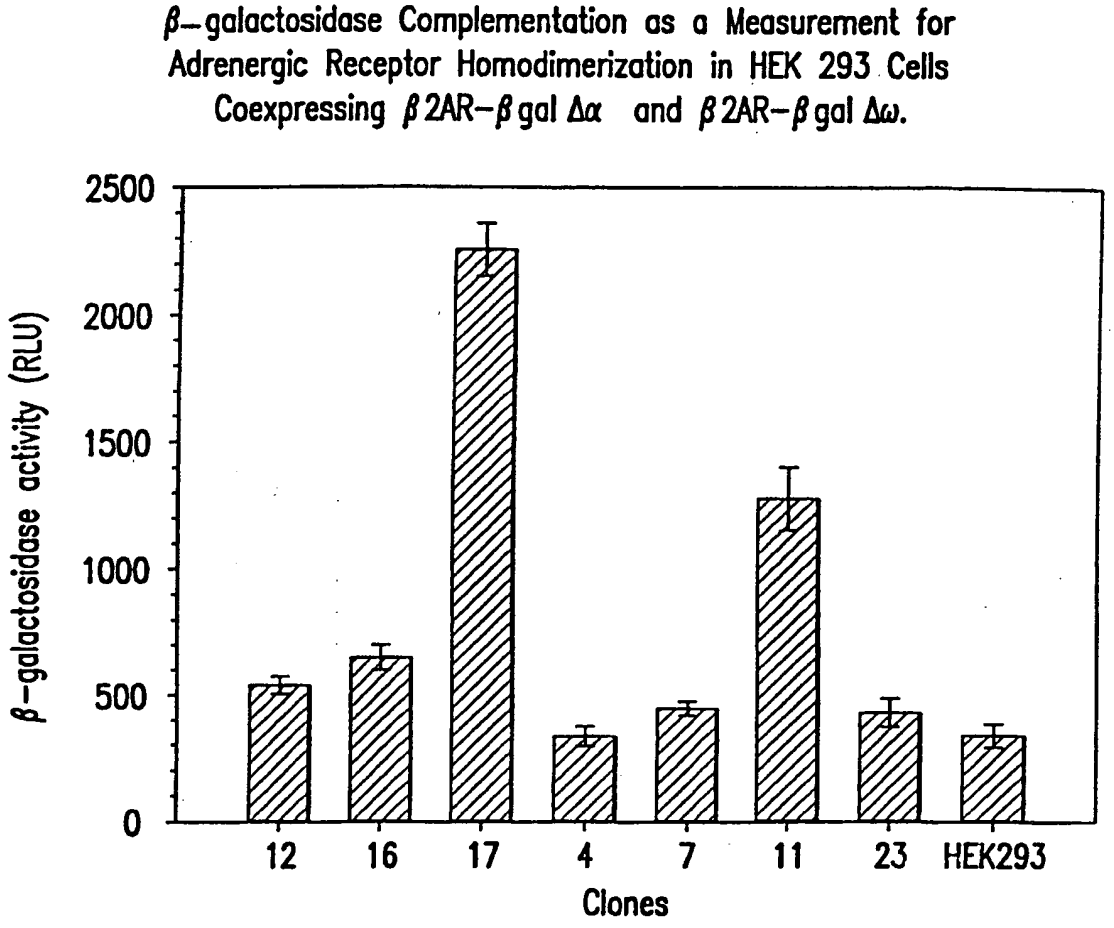
β-galactosidase Complementation as a Measurement for Adrenergic Receptor Homodimerization in HEK 293 Cells Coexpressing β2AR-βgal Δα and β2AR-βgal Δω.
β-galactosidase activity (RLU)
2500
2000
1500
1000
500
0
12
16
17
4
7
11
23
HEK293
Clones

FIG. 9A

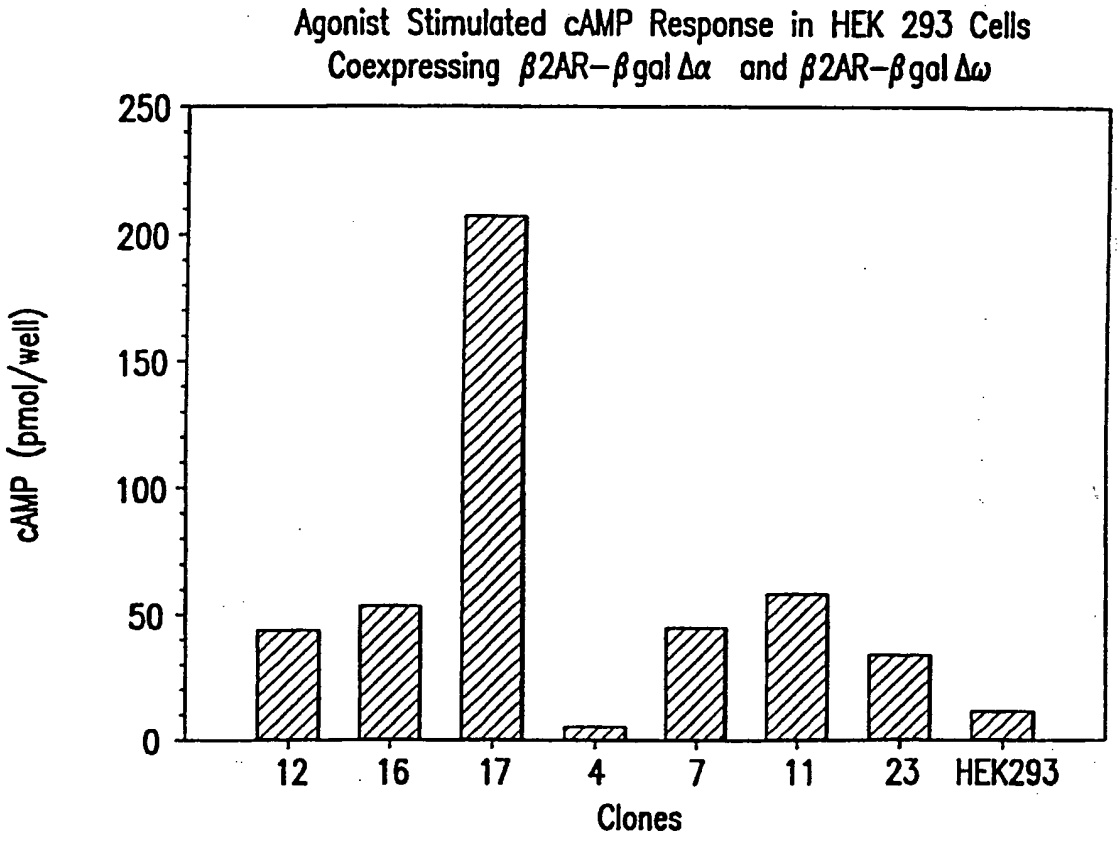
Agonist Stimulated cAMP Response in HEK 293 Cells
Coexpressing β2AR–βgal Δα and β2AR–βgal Δω
cAMP (pmol/well)
250
200
150
100
50
0
12
16
17
4
7
11
23
HEK293
Clones

FIG. 9B

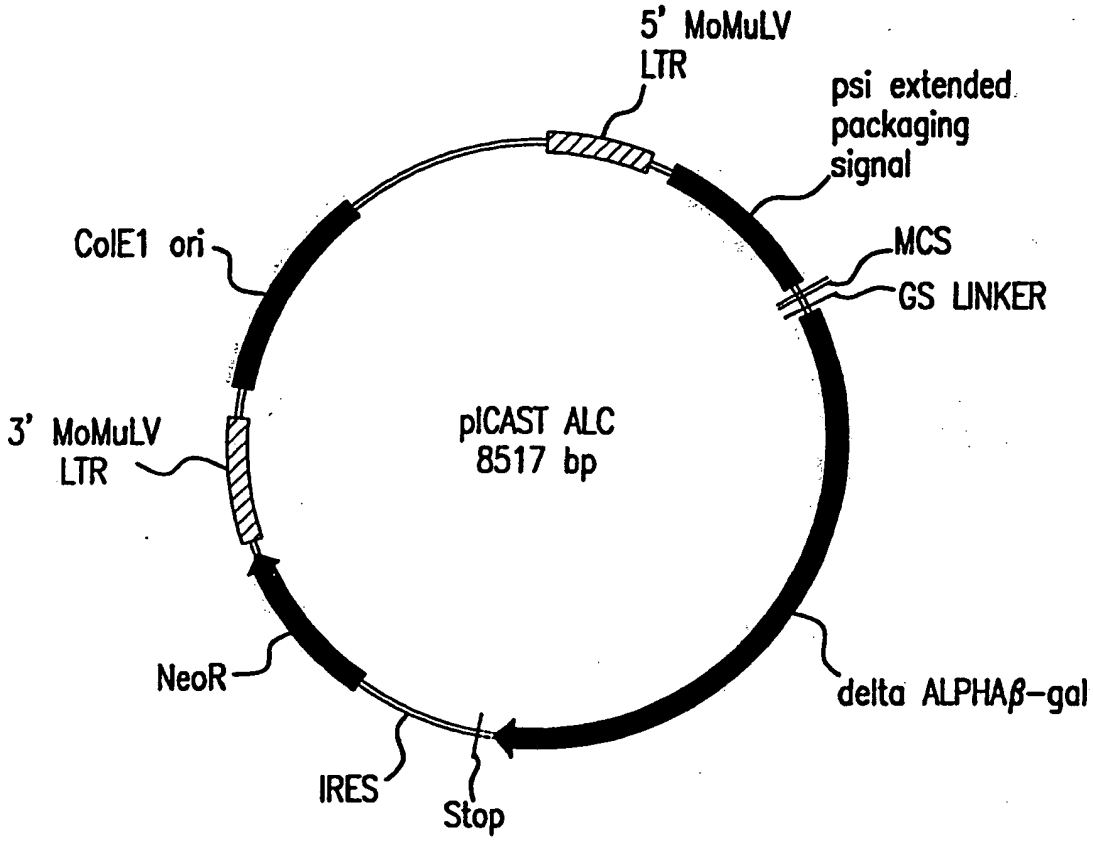
5' MoMuLV
LTR
psi extended
packaging
signal
MCS
GS LINKER
delta ALPHAβ-gal
Stop
IRES
NeoR
3' MoMuLV
LTR
ColE1 ori
pICAST ALC
8517 bp

FIG.10A

pICAST ALC

```
   1  CTGCAGCCTG AATATGGGCC AAACAGGATA TCTGTGGTAA GCAGTTCCTG
      GACGTCGGAC TTATACCCGG TTTGTCCTAT AGACACCATT CGTCAAGGAC

  51  CCCCGGCTCA GGGCCAAGAA CAGATGGAAC AGCTGAATAT GGGCCAAACA
      GGGGCCGAGT CCCGGTTCTT GTCTACCTTG TCGACTTATA CCCGGTTTGT

 101  GGATATCTGT GGTAAGCAGT TCCTGCCCCG GCTCAGGGCC AAGAACAGAT
      CCTATAGACA CCATTCGTCA AGGACGGGGC CGAGTCCCGG TTCTTGTCTA

 151  GGTCCCCAGA TGCGGTCCAG CCCTCAGCAG TTTCTAGAGA ACCATCAGAT
      CCAGGGGTCT ACGCCAGGTC GGGAGTCGTC AAAGATCTCT TGGTAGTCTA

 201  GTTTCCAGGG TGCCCCAAGG ACCTGAAATG ACCCTGTGCC TTATTTGAAC
      CAAAGGTCCC ACGGGGTTCC TGGACTTTAC TGGGACACGG AATAAACTTG

 251  TAACCAATCA GTTCGCTTCT CGCTTCTGTT CGCGCGCTTC TGCTCCCCGA
      ATTGGTTAGT CAAGCGAAGA GCGAAGACAA GCGCGCGAAG ACGAGGGGCT

 301  GCTCAATAAA AGAGCCCACA ACCCCTCACT CGGGGCGCCA GTCCTCCGAT
      CGAGTTATTT TCTCGGGTGT TGGGGAGTGA GCCCCGCGGT CAGGAGGCTA

 351  TGACTGAGTC GCCCGGGTAC CCGTGTATCC AATAAACCCT CTTGCAGTTG
      ACTGACTCAG CGGGCCCATG GGCACATAGG TTATTTGGGA GAACGTCAAC

 401  CATCCGACTT GTGGTCTCGC TGTTCCTTGG GAGGGTCTCC TCTGAGTGAT
      GTAGGCTGAA CACCAGAGCG ACAAGGAACC CTCCCAGAGG AGACTCACTA

 451  TGACTACCCG TCAGCGGGGG TCTTTCATTT GGGGGCTCGT CCGGGATCGG
      ACTGATGGGC AGTCGCCCCC AGAAAGTAAA CCCCCGAGCA GGCCCTAGCC

 501  GAGACCCCTG CCCAGGGACC ACCGACCCAC CACCGGGAGG CAAGCTGGCC
      CTCTGGGGAC GGGTCCCTGG TGGCTGGGTG GTGGCCCTCC GTTCGACCGG

 551  AGCAACTTAT CTGTGTCTGT CCGATTGTCT AGTGTCTATG ACTGATTTTA
      TCGTTGAATA GACACAGACA GGCTAACAGA TCACAGATAC TGACTAAAAT

 601  TGCGCCTGCG TCGGTACTAG TTAGCTAACT AGCTCTGTAT CTGGCGGACC
      ACGCGGACGC AGCCATGATC AATCGATTGA TCGAGACATA GACCGCCTGG
```

FIG.10B-1

pICAST ALC

651 CGTGGTGGAA CTGACGAGTT CTGAACACCC GGCCGCAACC CTGGGAGACG
GCACCACCTT GACTGCTCAA GACTTGTGGG CCGGCGTTGG GACCCTCTGC

701 TCCCAGGGAC TTTGGGGGCC GTTTTTGTGG CCCGACCTGA GGAAGGGAGT
AGGGTCCCTG AAACCCCCGG CAAAAACACC GGGCTGGACT CCTTCCCTCA

751 CGATGTGGAA TCCGACCCCG TCAGGATATG TGGTTCTGGT AGGAGACGAG
GCTACACCTT AGGCTGGGGC AGTCCTATAC ACCAAGACCA TCCTCTGCTC

801 AACCTAAAAC AGTTCCCGCC TCCGTCTGAA TTTTTGCTTT CGGTTTGGAA
TTGGATTTTG TCAAGGGCGG AGGCAGACTT AAAAACGAAA GCCAAACCTT

851 CCGAAGCCGC GCGTCTTGTC TGCTGCAGCA TCGTTCTGTG TTGTCTCTGT
GGCTTCGGCG CGCAGAACAG ACGACGTCGT AGCAAGACAC AACAGAGACA

901 CTGACTGTGT TTCTGTATTT GTCTGAAAAT TAGGGCCAGA CTGTTACCAC
GACTGACACA AAGACATAAA CAGACTTTTA ATCCCGGTCT GACAATGGTG

951 TCCCTTAAGT TTGACCTTAG GTAACTGGAA AGATGTCGAG CGGCTCGCTC
AGGGAATTCA AACTGGAATC CATTGACCTT TCTACAGCTC GCCGAGCGAG

1001 ACAACCAGTC GGTAGATGTC AAGAAGAGAC GTTGGGTTAC CTTCTGCTCT
TGTTGGTCAG CCATCTACAG TTCTTCTCTG CAACCCAATG GAAGACGAGA

1051 GCAGAATGGC CAACCTTTAA CGTCGGATGG CCGCGAGACG GCACCTTTAA
CGTCTTACCG GTTGGAAATT GCAGCCTACC GGCGCTCTGC CGTGGAAATT

1101 CCGAGACCTC ATCACCCAGG TTAAGATCAA GGTCTTTTCA CCTGGCCCGC
GGCTCTGGAG TAGTGGGTCC AATTCTAGTT CCAGAAAAGT GGACCGGGCG

1151 ATGGACACCC AGACCAGGTC CCCTACATCG TGACCTGGGA AGCCTTGGCT
TACCTGTGGG TCTGGTCCAG GGGATGTAGC ACTGGACCCT TCGGAACCGA

1201 TTTGACCCCC CTCCCTGGGT CAAGCCCTTT GTACACCCTA AGCCTCCGCC
AAACTGGGGG GAGGGACCCA GTTCGGGAAA CATGTGGGAT TCGGAGGCGG

1251 TCCTCTTCCT CCATCCGCCC CGTCTCTCCC CCTTGAACCT CCTCGTTCGA
AGGAGAAGGA GGTAGGCGGG GCAGAGAGGG GGAACTTGGA GGAGCAAGCT

FIG.10B-2

pICAST ALC

```
1301  CCCCGCCTCG ATCCTCCCTT TATCCAGCCC TCACTCCTTC TCTAGGCGCC
      GGGGCGGAGC TAGGAGGGAA ATAGGTCGGG AGTGAGGAAG AGATCCGCGG

1351  GGCCGCTCTA GCCCATTAAT ACGACTCACT ATAGGGCGAT TCGAATCAGG
      CCGGCGAGAT CGGGTAATTA TGCTGAGTGA TATCCCGCTA AGCTTAGTCC

1401  CCTTGGCGCG CCGGATCCTT AATTAAGCGC AATTGGGAGG TGGCGGTAGC
      GGAACCGCGC GGCCTAGGAA TTAATTCGCG TTAACCCTCC ACCGCCATCG

+2          M  G  V  I  T  D  S  L  A  V  V  A  R  T  D
           ]-------------------------------------------
1451  CTCGAGATGG GCGTGATTAC GGATTCACTG GCCGTCGTGG CCCGCACCGA
      GAGCTCTACC CGCACTAATG CCTAAGTGAC CGGCAGCACC GGGCGTGGCT

+2       R  P  S  Q  Q  L  R  S  L  N  G  E  W  R  F  A
         -------------------------------------------------
1501  TCGCCCTTCC CAACAGTTAC GCAGCCTGAA TGGCGAATGG CGCTTTGCCT
      AGCGGGAAGG GTTGTCAATG CGTCGGACTT ACCGCTTACC GCGAAACGGA

+2    W  F  P  A  P  E  A  V  P  E  S  W  L  E  C  D  L
      -------------------------------------------------
1551  GGTTTCCGGC ACCAGAAGCG GTGCCGGAAA GCTGGCTGGA GTGCGATCTT
      CCAAAGGCCG TGGTCTTCGC CACGGCCTTT CGACCGACCT CACGCTAGAA

+2     P  E  A  D  T  V  V  V  P  S  N  W  Q  M  H  G  Y
       -------------------------------------------------
1601  CCTGAGGCCG ATACTGTCGT CGTCCCCTCA AACTGGCAGA TGCACGGTTA
      GGACTCCGGC TATGACAGCA GCAGGGGAGT TTGACCGTCT ACGTGCCAAT

+2       D  A  P  I  Y  T  N  V  T  Y  P  I  T  V  N  P
         -------------------------------------------------
1651  CGATGCGCCC ATCTACACCA ACGTGACCTA TCCCATTACG GTCAATCCGC
      GCTACGCGGG TAGATGTGGT TGCACTGGAT AGGGTAATGC CAGTTAGGCG

+2    P  F  V  P  T  E  N  P  T  G  C  Y  S  L  T  F  N
      -------------------------------------------------
1701  CGTTTGTTCC CACGGAGAAT CCGACGGGTT GTTACTCGCT CACATTTAAT
      GCAAACAAGG GTGCCTCTTA GGCTGCCCAA CAATGAGCGA GTGTAAATTA
```

FIG.10B-3

pICAST ALC 21/78

```
+2      V  D  E  S  W  L  Q  E  G  Q  T  R  I  I  F  D  G
        ----------------------------------------------------
1751    GTTGATGAAA GCTGGCTACA GGAAGGCCAG ACGCGAATTA TTTTTGATGG
        CAACTACTTT CGACCGATGT CCTTCCGGTC TGCGCTTAAT AAAAACTACC

+2       V  N  S  A  F  H  L  W  C  N  G  R  W  V  G  Y
         ----------------------------------------------------
1801    CGTTAACTCG GCGTTTCATC TGTGGTGCAA CGGGCGCTGG GTCGGTTACG
        GCAATTGAGC CGCAAAGTAG ACACCACGTT GCCCGCGACC CAGCCAATGC

+2      G  Q  D  S  R  L  P  S  E  F  D  L  S  A  F  L  R
        ----------------------------------------------------
1851    GCCAGGACAG TCGTTTGCCG TCTGAATTTG ACCTGAGCGC ATTTTTACGC
        CGGTCCTGTC AGCAAACGGC AGACTTAAAC TGGACTCGCG TAAAAATGCG

+2      A  G  E  N  R  L  A  V  M  V  L  R  W  S  D  G  S
        ----------------------------------------------------
1901    GCCGGAGAAA ACCGCCTCGC GGTGATGGTG CTGCGCTGGA GTGACGGCAG
        CGGCCTCTTT TGGCGGAGCG CCACTACCAC GACGCGACCT CACTGCCGTC

+2       Y  L  E  D  Q  D  M  W  R  M  S  G  I  F  R  D
         ----------------------------------------------------
1951    TTATCTGGAA GATCAGGATA TGTGGCGGAT GAGCGGCATT TTCCGTGACG
        AATAGACCTT CTAGTCCTAT ACACCGCCTA CTCGCCGTAA AAGGCACTGC

+2      V  S  L  L  H  K  P  T  T  Q  I  S  D  F  H  V  A
        ----------------------------------------------------
2001    TCTCGTTGCT GCATAAACCG ACTACACAAA TCAGCGATTT CCATGTTGCC
        AGAGCAACGA CGTATTTGGC TGATGTGTTT AGTCGCTAAA GGTACAACGG

+2      T  R  F  N  D  D  F  S  R  A  V  L  E  A  E  V  Q
        ----------------------------------------------------
2051    ACTCGCTTTA ATGATGATTT CAGCCGCGCT GTACTGGAGG CTGAAGTTCA
        TGAGCGAAAT TACTACTAAA GTCGGCGCGA CATGACCTCC GACTTCAAGT
```

FIG.10B-4

pICAST ALC

```
+2      M  C  G  E  L  R  D  Y  L  R  V  T  V  S  L  W
        ------------------------------------------------
2101    GATGTGCGGC GAGTTGCGTG ACTACCTACG GGTAACAGTT TCTTTATGGC
        CTACACGCCG CTCAACGCAC TGATGGATGC CCATTGTCAA AGAAATACCG

+2      Q  G  E  T  Q  V  A  S  G  T  A  P  F  G  G  E  I
        ------------------------------------------------
2151    AGGGTGAAAC GCAGGTCGCC AGCGGCACCG CGCCTTTCGG CGGTGAAATT
        TCCCACTTTG CGTCCAGCGG TCGCCGTGGC GCGGAAAGCC GCCACTTTAA

+2      I  D  E  R  G  G  Y  A  D  R  V  T  L  R  L  N  V
        ------------------------------------------------
2201    ATCGATGAGC GTGGTGGTTA TGCCGATCGC GTCACACTAC GTCTGAACGT
        TAGCTACTCG CACCACCAAT ACGGCTAGCG CAGTGTGATG CAGACTTGCA

+2      E  N  P  K  L  W  S  A  E  I  P  N  L  Y  R  A
        ------------------------------------------------
2251    CGAAAACCCG AAACTGTGGA GCGCCGAAAT CCCGAATCTC TATCGTGCGG
        GCTTTTGGGC TTTGACACCT CGCGGCTTTA GGGCTTAGAG ATAGCACGCC

+2      V  V  E  L  H  T  A  D  G  T  L  I  E  A  E  A  C
        ------------------------------------------------
2301    TGGTTGAACT GCACACCGCC GACGGCACGC TGATTGAAGC AGAAGCCTGC
        ACCAACTTGA CGTGTGGCGG CTGCCGTGCG ACTAACTTCG TCTTCGGACG

+2      D  V  G  F  R  E  V  R  I  E  N  G  L  L  L  L  N
        ------------------------------------------------
2351    GATGTCGGTT TCCGCGAGGT GCGGATTGAA AATGGTCTGC TGCTGCTGAA
        CTACAGCCAA AGGCGCTCCA CGCCTAACTT TTACCAGACG ACGACGACTT

+2      G  K  P  L  L  I  R  G  V  N  R  H  E  H  H  P
        ------------------------------------------------
2401    CGGCAAGCCG TTGCTGATTC GAGGCGTTAA CCGTCACGAG CATCATCCTC
        GCCGTTCGGC AACGACTAAG CTCCGCAATT GGCAGTGCTC GTAGTAGGAG
```

FIG.10B-5

pICAST ALC

```
+2     L  H  G  Q  V  M  D  E  Q  T  M  V  Q  D  I  L  L
       ----------------------------------------------------
2451   TGCATGGTCA GGTCATGGAT GAGCAGACGA TGGTGCAGGA TATCCTGCTG
       ACGTACCAGT CCAGTACCTA CTCGTCTGCT ACCACGTCCT ATAGGACGAC

+2      M  K  Q  N  N  F  N  A  V  R  C  S  H  Y  P  N  H
       ----------------------------------------------------
2501   ATGAAGCAGA ACAACTTTAA CGCCGTGCGC TGTTCGCATT ATCCGAACCA
       TACTTCGTCT TGTTGAAATT GCGGCACGCG ACAAGCGTAA TAGGCTTGGT

+2        P  L  W  Y  T  L  C  D  R  Y  G  L  Y  V  V  D
       ----------------------------------------------------
2551   TCCGCTGTGG TACACGCTGT GCGACCGCTA CGGCCTGTAT GTGGTGGATG
       AGGCGACACC ATGTGCGACA CGCTGGCGAT GCCGGACATA CACCACCTAC

+2     E  A  N  I  E  T  H  G  M  V  P  M  N  R  L  T  D
       ----------------------------------------------------
2601   AAGCCAATAT TGAAACCCAC GGCATGGTGC CAATGAATCG TCTGACCGAT
       TTCGGTTATA ACTTTGGGTG CCGTACCACG GTTACTTAGC AGACTGGCTA

+2      D  P  R  W  L  P  A  M  S  E  R  V  T  R  M  V  Q
       ----------------------------------------------------
2651   GATCCGCGCT GGCTACCGGC GATGAGCGAA CGCGTAACGC GAATGGTGCA
       CTAGGCGCGA CCGATGGCCG CTACTCGCTT GCGCATTGCG CTTACCACGT

+2        R  D  R  N  H  P  S  V  I  I  W  S  L  G  N  E
       ----------------------------------------------------
2701   GCGCGATCGT AATCACCCGA GTGTGATCAT CTGGTCGCTG GGGAATGAAT
       CGCGCTAGCA TTAGTGGGCT CACACTAGTA GACCAGCGAC CCCTTACTTA

+2     S  G  H  G  A  N  H  D  A  L  Y  R  W  I  K  S  V
       ----------------------------------------------------
2751   CAGGCCACGG CGCTAATCAC GACGCGCTGT ATCGCTGGAT CAAATCTGTC
       GTCCGGTGCC GCGATTAGTG CTGCGCGACA TAGCGACCTA GTTTAGACAG
```

FIG.10B-6

pICAST ALC

```
+2      D  P  S  R  P  V  Q  Y  E  G  G  G  A  D  T  T  A
        ------------------------------------------------
2801    GATCCTTCCC GCCCGGTGCA GTATGAAGGC GGCGGAGCCG ACACCACGGC
        CTAGGAAGGG CGGGCCACGT CATACTTCCG CCGCCTCGGC TGTGGTGCCG

+2        T  D  I  I  C  P  M  Y  A  R  V  D  E  D  Q  P
        ------------------------------------------------
2851    CACCGATATT ATTTGCCCGA TGTACGCGCG CGTGGATGAA GACCAGCCCT
        GTGGCTATAA TAAACGGGCT ACATGCGCGC GCACCTACTT CTGGTCGGGA

+2      F  P  A  V  P  K  W  S  I  K  K  W  L  S  L  P  G
        ------------------------------------------------
2901    TCCCGGCTGT GCCGAAATGG TCCATCAAAA AATGGCTTTC GCTACCTGGA
        AGGGCCGACA CGGCTTTACC AGGTAGTTTT TTACCGAAAG CGATGGACCT

+2      E  T  R  P  L  I  L  C  E  Y  A  H  A  M  G  N  S
        ------------------------------------------------
2951    GAGACGCGCC CGCTGATCCT TTGCGAATAC GCCCACGCGA TGGGTAACAG
        CTCTGCGCGG GCGACTAGGA AACGCTTATG CGGGTGCGCT ACCCATTGTC

+2        L  G  G  F  A  K  Y  W  Q  A  F  R  Q  Y  P  R
        ------------------------------------------------
3001    TCTTGGCGGT TTCGCTAAAT ACTGGCAGGC GTTTCGTCAG TATCCCCGTT
        AGAACCGCCA AAGCGATTTA TGACCGTCCG CAAAGCAGTC ATAGGGGCAA

+2      L  Q  G  G  F  V  W  D  W  V  D  Q  S  L  I  K  Y
        ------------------------------------------------
3051    TACAGGGCGG CTTCGTCTGG GACTGGGTGG ATCAGTCGCT GATTAAATAT
        ATGTCCCGCC GAAGCAGACC CTGACCCACC TAGTCAGCGA CTAATTTATA

+2      D  E  N  G  N  P  W  S  A  Y  G  G  D  F  G  D  T
        ------------------------------------------------
3101    GATGAAAACG GCAACCCGTG GTCGGCTTAC GGCGGTGATT TTGGCGATAC
        CTACTTTTGC CGTTGGGCAC CAGCCGAATG CCGCCACTAA AACCGCTATG
```

FIG.10B-7

pICAST ALC

```
+2      P  N  D  R  Q  F  C  M  N  G  L  V  F  A  D  R
        ------------------------------------------------
3151    GCCGAACGAT CGCCAGTTCT GTATGAACGG TCTGGTCTTT GCCGACCGCA
        CGGCTTGCTA GCGGTCAAGA CATACTTGCC AGACCAGAAA CGGCTGGCGT

+2      T  P  H  P  A  L  T  E  A  K  H  Q  Q  Q  F  F  Q
        ------------------------------------------------
3201    CGCCGCATCC AGCGCTGACG GAAGCAAAAC ACCAGCAGCA GTTTTTCCAG
        GCGGCGTAGG TCGCGACTGC CTTCGTTTTG TGGTCGTCGT CAAAAAGGTC

+2      F  R  L  S  G  Q  T  I  E  V  T  S  E  Y  L  F  R
        ------------------------------------------------
3251    TTCCGTTTAT CCGGGCAAAC CATCGAAGTG ACCAGCGAAT ACCTGTTCCG
        AAGGCAAATA GGCCCGTTTG GTAGCTTCAC TGGTCGCTTA TGGACAAGGC

+2      H  S  D  N  E  L  L  H  W  M  V  A  L  D  G  K
        ------------------------------------------------
3301    TCATAGCGAT AACGAGCTCC TGCACTGGAT GGTGGCGCTG GATGGTAAGC
        AGTATCGCTA TTGCTCGAGG ACGTGACCTA CCACCGCGAC CTACCATTCG

+2      P  L  A  S  G  E  V  P  L  D  V  A  P  Q  G  K  Q
        ------------------------------------------------
3351    CGCTGGCAAG CGGTGAAGTG CCTCTGGATG TCGCTCCACA AGGTAAACAG
        GCGACCGTTC GCCACTTCAC GGAGACCTAC AGCGAGGTGT TCCATTTGTC

+2      L  I  E  L  P  E  L  P  Q  P  E  S  A  G  Q  L  W
        ------------------------------------------------
3401    TTGATTGAAC TGCCTGAACT ACCGCAGCCG GAGAGCGCCG GGCAACTCTG
        AACTAACTTG ACGGACTTGA TGGCGTCGGC CTCTCGCGGC CCGTTGAGAC

+2      L  T  V  R  V  V  Q  P  N  A  T  A  W  S  E  A
        ------------------------------------------------
3451    GCTCACAGTA CGCGTAGTGC AACCGAACGC GACCGCATGG TCAGAAGCCG
        CGAGTGTCAT GCGCATCACG TTGGCTTGCG CTGGCGTACC AGTCTTCGGC
```

FIG.10B-8

pICAST ALC

```
+2     G  H  I  S  A  W  Q  Q  W  R  L  A  E  N  L  S  V
       ------------------------------------------------
3501   GGCACATCAG CGCCTGGCAG CAGTGGCGTC TGGCGGAAAA CCTCAGTGTG
       CCGTGTAGTC GCGGACCGTC GTCACCGCAG ACCGCCTTTT GGAGTCACAC

+2      T  L  P  A  A  S  H  A  I  P  H  L  T  T  S  E  M
       ------------------------------------------------
3551   ACGCTCCCCG CCGCGTCCCA CGCCATCCCG CATCTGACCA CCAGCGAAAT
       TGCGAGGGGC GGCGCAGGGT GCGGTAGGGC GTAGACTGGT GGTCGCTTTA

+2       D  F  C  I  E  L  G  N  K  R  W  Q  F  N  R  Q
       ------------------------------------------------
3601   GGATTTTTGC ATCGAGCTGG GTAATAAGCG TTGGCAATTT AACCGCCAGT
       CCTAAAAACG TAGCTCGACC CATTATTCGC AACCGTTAAA TTGGCGGTCA

+2     S  G  F  L  S  Q  M  W  I  G  D  K  K  Q  L  L  T
       ------------------------------------------------
3651   CAGGCTTTCT TTCACAGATG TGGATTGGCG ATAAAAAACA ACTGCTGACG
       GTCCGAAAGA AAGTGTCTAC ACCTAACCGC TATTTTTTGT TGACGACTGC

+2      P  L  R  D  Q  F  T  R  A  P  L  D  N  D  I  G  V
       ------------------------------------------------
3701   CCGCTGCGCG ATCAGTTCAC CCGTGCACCG CTGGATAACG ACATTGGCGT
       GGCGACGCGC TAGTCAAGTG GGCACGTGGC GACCTATTGC TGTAACCGCA

+2       S  E  A  T  R  I  D  P  N  A  W  V  E  R  W  K
       ------------------------------------------------
3751   AAGTGAAGCG ACCCGCATTG ACCCTAACGC CTGGGTCGAA CGCTGGAAGG
       TTCACTTCGC TGGGCGTAAC TGGGATTGCG GACCCAGCTT GCGACCTTCC

+2     A  A  G  H  Y  Q  A  E  A  A  L  L  Q  C  T  A  D
       ------------------------------------------------
3801   CGGCGGGCCA TTACCAGGCC GAAGCAGCGT TGTTGCAGTG CACGGCAGAT
       GCCGCCCGGT AATGGTCCGG CTTCGTCGCA ACAACGTCAC GTGCCGTCTA
```

FIG.10B-9

pICAST ALC

```
+2      T  L  A  D  A  V  L  I  T  T  A  H  A  W  Q  H  Q
        ------------------------------------------------
3851    ACACTTGCTG ATGCGGTGCT GATTACGACC GCTCACGCGT GGCAGCATCA
        TGTGAACGAC TACGCCACGA CTAATGCTGG CGAGTGCGCA CCGTCGTAGT

+2       G  K  T  L  F  I  S  R  K  T  Y  R  I  D  G  S
         ---------------------------------------------
3901    GGGGAAAACC TTATTTATCA GCCGGAAAAC CTACCGGATT GATGGTAGTG
        CCCCTTTTGG AATAAATAGT CGGCCTTTTG GATGGCCTAA CTACCATCAC

+2      G  Q  M  A  I  T  V  D  V  E  V  A  S  D  T  P  H
        ------------------------------------------------
3951    GTCAAATGGC GATTACCGTT GATGTTGAAG TGGCGAGCGA TACACCGCAT
        CAGTTTACCG CTAATGGCAA CTACAACTTC ACCGCTCGCT ATGTGGCGTA

+2      P  A  R  I  G  L  N  C  Q  L  A  Q  V  A  E  R  V
        ------------------------------------------------
4001    CCGGCGCGGA TTGGCCTGAA CTGCCAGCTG GCGCAGGTAG CAGAGCGGGT
        GGCCGCGCCT AACCGGACTT GACGGTCGAC CGCGTCCATC GTCTCGCCCA

+2       N  W  L  G  L  G  P  Q  E  N  Y  P  D  R  L  T
         ---------------------------------------------
4051    AAACTGGCTC GGATTAGGGC CGCAAGAAAA CTATCCCGAC CGCCTTACTG
        TTTGACCGAG CCTAATCCCG GCGTTCTTTT GATAGGGCTG GCGGAATGAC

+2      A  A  C  F  D  R  W  D  L  P  L  S  D  M  Y  T  P
        ------------------------------------------------
4101    CCGCCTGTTT TGACCGCTGG GATCTGCCAT TGTCAGACAT GTATACCCCG
        GGCGGACAAA ACTGGCGACC CTAGACGGTA ACAGTCTGTA CATATGGGGC

+2      T  V  F  P  S  E  N  G  L  R  C  G  T  R  E  L  N
        ------------------------------------------------
4151    TACGTCTTCC CGAGCGAAAA CGGTCTGCGC TGCGGGACGC GCGAATTGAA
        ATGCAGAAGG GCTCGCTTTT GCCAGACGCG ACGCCCTGCG CGCTTAACTT
```

FIG.10B-10

pICAST ALC

```
+2      Y  G  P  H  Q  W  R  G  D  F  Q  F  N  I  S  R
        ------------------------------------------------
4201    TTATGGCCCA CACCAGTGGC GCGGCGACTT CCAGTTCAAC ATCAGCCGCT
        AATACCGGGT GTGGTCACCG CGCCGCTGAA GGTCAAGTTG TAGTCGGCGA

+2      Y  S  Q  Q  Q  L  M  E  T  S  H  R  H  L  L  H  A
        ------------------------------------------------
4251    ACAGTCAACA GCAACTGATG GAAACCAGCC ATCGCCATCT GCTGCACGCG
        TGTCAGTTGT CGTTGACTAC CTTTGGTCGG TAGCGGTAGA CGACGTGCGC

+2      E  E  G  T  W  L  N  I  D  G  F  H  M  G  I  G  G
        ------------------------------------------------
4301    GAAGAAGGCA CATGGCTGAA TATCGACGGT TTCCATATGG GGATTGGTGG
        CTTCTTCCGT GTACCGACTT ATAGCTGGCA AAGGTATACC CCTAACCACC

+2      D  D  S  W  S  P  S  V  S  A  E  F  Q  L  S  A
        ------------------------------------------------
4351    CGACGACTCC TGGAGCCCGT CAGTATCGGC GGAATTCCAG CTGAGCGCCG
        GCTGCTGAGG ACCTCGGGCA GTCATAGCCG CCTTAAGGTC GACTCGCGGC

+2      G  R  Y  H  Y  Q  L  V  W  C  Q  K  R  S  D  Y  K
        ------------------------------------------------
4401    GTCGCTACCA TTACCAGTTG GTCTGGTGTC AAAAAAGATC TGACTATAAA
        CAGCGATGGT AATGGTCAAC CAGACCACAG TTTTTTCTAG ACTGATATTT

+2      D  E  D  L  D  H  H  H  H  H  H  R
        ---------------------------------->
4451    GATGAGGACC TCGACCATCA TCATCATCAT CACCGGTAAT AATAGGTAGA
        CTACTCCTGG AGCTGGTAGT AGTAGTAGTA GTGGCCATTA TTATCCATCT

4501    TAAGTGACTG ATTAGATGCA TTGATCCCTC GACCAATTCC GGTTATTTTC
        ATTCACTGAC TAATCTACGT AACTAGGGAG CTGGTTAAGG CCAATAAAAG

4551    CACCATATTG CCGTCTTTTG GCAATGTGAG GGCCCGGAAA CCTGGCCCTG
        GTGGTATAAC GGCAGAAAAC CGTTACACTC CCGGGCCTTT GGACCGGGAC
```

FIG.10B-11

pICAST ALC

4601 TCTTCTTGAC GAGCATTCCT AGGGGTCTTT CCCCTCTCGC CAAAGGAATG
AGAAGAACTG CTCGTAAGGA TCCCCAGAAA GGGGAGAGCG GTTTCCTTAC

4651 CAAGGTCTGT TGAATGTCGT GAAGGAAGCA GTTCCTCTGG AAGCTTCTTG
GTTCCAGACA ACTTACAGCA CTTCCTTCGT CAAGGAGACC TTCGAAGAAC

4701 AAGACAAACA ACGTCTGTAG CGACCCTTTG CAGGCAGCGG AACCCCCCAC
TTCTGTTTGT TGCAGACATC GCTGGGAAAC GTCCGTCGCC TTGGGGGGTG

4751 CTGGCGACAG GTGCCTCTGC GGCCAAAAGC CACGTGTATA AGATACACCT
GACCGCTGTC CACGGAGACG CCGGTTTTCG GTGCACATAT TCTATGTGGA

4801 GCAAAGGCGG CACAACCCCA GTGCCACGTT GTGAGTTGGA TAGTTGTGGA
CGTTTCCGCC GTGTTGGGGT CACGGTGCAA CACTCAACCT ATCAACACCT

4851 AAGAGTCAAA TGGCTCTCCT CAAGCGTATT CAACAAGGGG CTGAAGGATG
TTCTCAGTTT ACCGAGAGGA GTTCGCATAA GTTGTTCCCC GACTTCCTAC

4901 CCCAGAAGGT ACCCCATTGT ATGGGATCTG ATCTGGGGCC TCGGTGCACA
GGGTCTTCCA TGGGGTAACA TACCCTAGAC TAGACCCCGG AGCCACGTGT

4951 TGCTTTACAT GTGTTTAGTC GAGGTTAAAA AACGTCTAGG CCCCCCGAAC
ACGAAATGTA CACAAATCAG CTCCAATTTT TTGCAGATCC GGGGGGCTTG

5001 CACGGGGACG TGGTTTTCCT TTGAAAAACA CGATGATAAT ACCATGATTG

GTGCCCCTGC ACCAAAAGGA AACTTTTTGT GCTACTATTA TGGTACTAAC

5051 AACAAGATGG ATTGCACGCA GGTTCTCCGG CCGCTTGGGT GGAGAGGCTA
TTGTTCTACC TAACGTGCGT CCAAGAGGCC GGCGAACCCA CCTCTCCGAT

5101 TTCGGCTATG ACTGGGCACA ACAGACAATC GGCTGCTCTG ATGCCGCCGT
AAGCCGATAC TGACCCGTGT TGTCTGTTAG CCGACGAGAC TACGGCGGCA

5151 GTTCCGGCTG TCAGCGCAGG GGCGCCCGGT TCTTTTTGTC AAGACCGACC
CAAGGCCGAC AGTCGCGTCC CCGCGGGCCA AGAAAAACAG TTCTGGCTGG

FIG.10B-12

pICAST ALC

```
5201  TGTCCGGTGC CCTGAATGAA CTGCAGGACG AGGCAGCGCG GCTATCGTGG
      ACAGGCCACG GGACTTACTT GACGTCCTGC TCCGTCGCGC CGATAGCACC

5251  CTGGCCACGA CGGGCGTTCC TTGCGCAGCT GTGCTCGACG TTGTCACTGA
      GACCGGTGCT GCCCGCAAGG AACGCGTCGA CACGAGCTGC AACAGTGACT

5301  AGCGGGAAGG GACTGGCTGC TATTGGGCGA AGTGCCGGGG CAGGATCTCC
      TCGCCCTTCC CTGACCGACG ATAACCCGCT TCACGGCCCC GTCCTAGAGG

5351  TGTCATCTCA CCTTGCTCCT GCCGAGAAAG TATCCATCAT GGCTGATGCA
      ACAGTAGAGT GGAACGAGGA CGGCTCTTTC ATAGGTAGTA CCGACTACGT

5401  ATGCGGCGGC TGCATACGCT TGATCCGGCT ACCTGCCCAT TCGACCACCA
      TACGCCGCCG ACGTATGCGA ACTAGGCCGA TGGACGGGTA AGCTGGTGGT

5451  AGCGAAACAT CGCATCGAGC GAGCACGTAC TCGGATGGAA GCCGGTCTTG
      TCGCTTTGTA GCGTAGCTCG CTCGTGCATG AGCCTACCTT CGGCCAGAAC

5501  TCGATCAGGA TGATCTGGAC GAAGAGCATC AGGGGCTCGC GCCAGCCGAA
      AGCTAGTCCT ACTAGACCTG CTTCTCGTAG TCCCCGAGCG CGGTCGGCTT

5551  CTGTTCGCCA GGCTCAAGGC GCGCATGCCC GACGGCGAGG ATCTCGTCGT
      GACAAGCGGT CCGAGTTCCG CGCGTACGGG CTGCCGCTCC TAGAGCAGCA

5601  GACCCATGGC GATGCCTGCT TGCCGAATAT CATGGTGGAA AATGGCCGCT
      CTGGGTACCG CTACGGACGA ACGGCTTATA GTACCACCTT TTACCGGCGA

5651  TTTCTGGATT CATCGACTGT GGCCGGCTGG GTGTGGCGGA CCGCTATCAG
      AAAGACCTAA GTAGCTGACA CCGGCCGACC CACACCGCCT GGCGATAGTC

5701  GACATAGCGT TGGCTACCCG TGATATTGCT GAAGAGCTTG GCGGCGAATG
      CTGTATCGCA ACCGATGGGC ACTATAACGA CTTCTCGAAC CGCCGCTTAC

5751  GGCTGACCGC TTCCTCGTGC TTTACGGTAT CGCCGCTCCC GATTCGCAGC
      CCGACTGGCG AAGGAGCACG AAATGCCATA GCGGCGAGGG CTAAGCGTCG
```

FIG.10B-13

pICAST ALC

```
5801  GCATCGCCTT CTATCGCCTT CTTGACGAGT TCTTCTGAGC GGGACTCTGG
      CGTAGCGGAA GATAGCGGAA GAACTGCTCA AGAAGACTCG CCCTGAGACC

5851  GGTTCGCATC GATAAAATAA AAGATTTTAT TTAGTCTCCA GAAAAAGGGG
      CCAAGCGTAG CTATTTTATT TTCTAAAATA AATCAGAGGT CTTTTTCCCC

5901  GGAATGAAAG ACCCCACCTG TAGGTTTGGC AAGCTAGCTT AAGTAACGCC
      CCTTACTTTC TGGGGTGGAC ATCCAAACCG TTCGATCGAA TTCATTGCGG

5951  ATTTTGCAAG GCATGGAAAA ATACATAACT GAGAATAGAG AAGTTCAGAT
      TAAAACGTTC CGTACCTTTT TATGTATTGA CTCTTATCTC TTCAAGTCTA

6001  CAAGGTCAGG AACAGATGGA ACAGCTGAAT ATGGGCCAAA CAGGATATCT
      GTTCCAGTCC TTGTCTACCT TGTCGACTTA TACCCGGTTT GTCCTATAGA

6051  GTGGTAAGCA GTTCCTGCCC CGGCTCAGGG CCAAGAACAG ATGGAACAGC
      CACCATTCGT CAAGGACGGG GCCGAGTCCC GGTTCTTGTC TACCTTGTCG

6101  TGAATATGGG CCAAACAGGA TATCTGTGGT AAGCAGTTCC TGCCCCGGCT
      ACTTATACCC GGTTTGTCCT ATAGACACCA TTCGTCAAGG ACGGGGCCGA

6151  CAGGGCCAAG AACAGATGGT CCCCAGATGC GGTCCAGCCC TCAGCAGTTT
      GTCCCGGTTC TTGTCTACCA GGGGTCTACG CCAGGTCGGG AGTCGTCAAA

6201  CTAGAGAACC ATCAGATGTT TCCAGGGTGC CCCAAGGACC TGAAATGACC
      GATCTCTTGG TAGTCTACAA AGGTCCCACG GGGTTCCTGG ACTTTACTGG

6251  CTGTGCCTTA TTTGAACTAA CCAATCAGTT CGCTTCTCGC TTCTGTTCGC
      GACACGGAAT AAACTTGATT GGTTAGTCAA GCGAAGAGCG AAGACAAGCG

6301  GCGCTTCTGC TCCCCGAGCT CAATAAAAGA GCCCACAACC CCTCACTCGG
      CGCGAAGACG AGGGGCTCGA GTTATTTTCT CGGGTGTTGG GGAGTGAGCC

6351  GGCGCCAGTC CTCCGATTGA CTGAGTCGCC CGGGTACCCG TGTATCCAAT
      CCGCGGTCAG GAGGCTAACT GACTCAGCGG GCCCATGGGC ACATAGGTTA
```

FIG.10B-14

pICAST ALC

6401 AAACCCTCTT GCAGTTGCAT CCGACTTGTG GTCTCGCTGT TCCTTGGGAG
TTTGGGAGAA CGTCAACGTA GGCTGAACAC CAGAGCGACA AGGAACCCTC

6451 GGTCTCCTCT GAGTGATTGA CTACCCGTCA GCGGGGGTCT TTCATTCATG
CCAGAGGAGA CTCACTAACT GATGGGCAGT CGCCCCCAGA AAGTAAGTAC

6501 CAGCATGTAT CAAAATTAAT TTGGTTTTTT TTCTTAAGTA TTTACATTAA
GTCGTACATA GTTTTAATTA AACCAAAAAA AAGAATTCAT AAATGTAATT

6551 ATGGCCATAG TTGCATTAAT GAATCGGCCA ACGCGCGGGG AGAGGCGGTT
TACCGGTATC AACGTAATTA CTTAGCCGGT TGCGCGCCCC TCTCCGCCAA

6601 TGCGTATTGG CGCTCTTCCG CTTCCTCGCT CACTGACTCG CTGCGCTCGG
ACGCATAACC GCGAGAAGGC GAAGGAGCGA GTGACTGAGC GACGCGAGCC

6651 TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG
AGCAAGCCGA CGCCGCTCGC CATAGTCGAG TGAGTTTCCG CCATTATGCC

FIG.10B-15

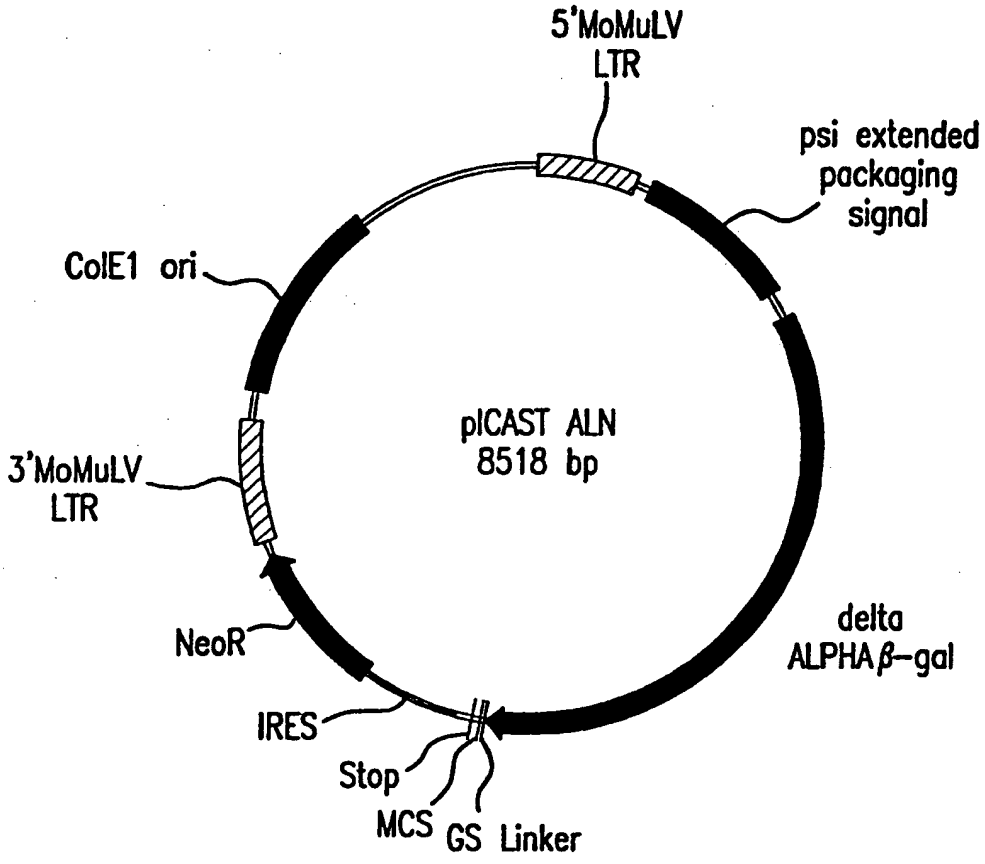
5'MoMuLV
LTR
psi extended
packaging
signal
ColE1 ori
pICAST ALN
8518 bp
3'MoMuLV
LTR
NeoR
delta
ALPHA β-gal
IRES
Stop
MCS
GS Linker

FIG.11A

pICAST ALN

```
CTGCAGCCTG AATATGGGCC AAACAGGATA TCTGTGGTAA GCAGTTCCTG CCCCGGCTCA     60
GACGTCGGAC TTATACCCGG TTTGTCCTAT AGACACCATT CGTCAAGGAC GGGGCCGAGT     60

GGGCCAAGAA CAGATGGAAC AGCTGAATAT GGGCCAAACA GGATATCTGT GGTAAGCAGT    120
CCCGGTTCTT GTCTACCTTG TCGACTTATA CCCGGTTTGT CCTATAGACA CCATTCGTCA    120

TCCTGCCCCG GCTCAGGGCC AAGAACAGAT GGTCCCCAGA TGCGGTCCAG CCCTCAGCAG    180
AGGACGGGGC CGAGTCCCGG TTCTTGTCTA CCAGGGGTCT ACGCCAGGTC GGGAGTCGTC    180

TTTCTAGAGA ACCATCAGAT GTTTCCAGGG TGCCCCAAGG ACCTGAAATG ACCCTGTGCC    240
AAAGATCTCT TGGTAGTCTA CAAAGGTCCC ACGGGGTTCC TGGACTTTAC TGGGACACGG    240

TTATTTGAAC TAACCAATCA GTTCGCTTCT CGCTTCTGTT CGCGCGCTTC TGCTCCCCGA    300
AATAAACTTG ATTGGTTAGT CAAGCGAAGA GCGAAGACAA GCGCGCGAAG ACGAGGGGCT    300

GCTCAATAAA AGAGCCCACA ACCCGTCACT CGGGGCGCCA GTCCTCCGAT TGACTGAGTC    360
CGAGTTATTT TCTCGGGTGT TGGGGAGTGA GCCCCGCGGT CAGGAGGCTA ACTGACTCAG    360

GCCCGGGTAC CCGTGTATCC AATAAACCCT CTTGCAGTTG CATCCGACTT GTGGTCTCGC    420
CGGGCCCATG GGCACATAGG TTATTTGGGA GAACGTCAAC GTAGGCTGAA CACCAGAGCG    420

TGTTCCTTGG GAGGGTCTCC TCTGAGTGAT TGACTACCCG TCAGCGGGGG TCTTTCATTT    480
ACAAGGAACC CTCCCAGAGG AGACTCACTA ACTGATGGGC AGTCGCCCCC AGAAAGTAAA    480

GGGGGCTCGT CCGGGATCGG GAGACCCCTG CCCAGGGACC ACCGACCCAC CACCGGGAGG    540
CCCCCGAGCA GGCCCTAGCC CTCTGGGGAC GGGTCCCTGG TGGCTGGGTG GTGGCCCTCC    540

CAAGCTGGCC AGCAACTTAT CTGTGTCTGT CCGATTGTCT AGTGTCTATG ACTGATTTTA    600
GTTCGACCGG TCGTTGAATA GACACAGACA GGCTAACAGA TCACAGATAC TGACTAAAAT    600

TGCGCCTGCG TCGGTACTAG TTAGCTAACT AGCTCTGTAT CTGGCGGACC CGTGGTGGAA    660
ACGCGGACGC AGCCATGATC AATCGATTGA TCGAGACATA GACCGCCTGG GCACCACCTT    660

CTGACGAGTT CTGAACACCC GGCCGCAACC CTGGGAGACG TCCCAGGGAC TTTGGGGGCC    720
GACTGCTCAA GACTTGTGGG CCGGCGTTGG GACCCTCTGC AGGGTCCCTG AAACCCCCGG    720

GTTTTTGTGG CCCGACCTGA GGAAGGGAGT CGATGTGGAA TCCGACCCCG TCAGGATATG    780
CAAAAACACC GGGCTGGACT CCTTCCCTCA GCTACACCTT AGGCTGGGGC AGTCCTATAC    780
```

FIG.11B-1

pICAST ALN

```
TGGTTCTGGT AGGAGACGAG AACCTAAAAC AGTTCCCGCC TCCGTCTGAA TTTTTGCTTT   840
ACCAAGACCA TCCTCTGCTC TTGGATTTTG TCAAGGGCGG AGGCAGACTT AAAAACGAAA   840

CGGTTTGGAA CCGAAGCCGC GCGTCTTGTC TGCTGCAGCA TCGTTCTGTG TTGTCTCTGT   900
GCCAAACCTT GGCTTCGGCG CGCAGAACAG ACGACGTCGT AGCAAGACAC AACAGAGACA   900

CTGACTGTGT TTCTGTATTT GTCTGAAAAT TAGGGCCAGA CTGTTACCAC TCCCTTAAGT   960
GACTGACACA AAGACATAAA CAGACTTTTA ATCCCGGTCT GACAATGGTG AGGGAATTCA   960

TTGACCTTAG GTAACTGGAA AGATGTCGAG CGGCTCGCTC ACAACCAGTC GGTAGATGTC  1020
AACTGGAATC CATTGACCTT TCTACAGCTC GCCGAGCGAG TGTTGGTCAG CCATCTACAG  1020

AAGAAGAGAC GTTGGGTTAC CTTCTGCTCT GCAGAATGGC CAACCTTTAA CGTCGGATGG  1080
TTCTTCTCTG CAACCCAATG GAAGACGAGA CGTCTTACCG GTTGGAAATT GCAGCCTACC  1080

CCGCGAGACG GCACCTTTAA CCGAGACCTC ATCACCCAGG TTAAGATCAA GGTCTTTTCA  1140
GGCGCTCTGC CGTGGAAATT GGCTCTGGAG TAGTGGGTCC AATTCTAGTT CCAGAAAAGT  1140

CCTGGCCCGC ATGGACACCC AGACCAGGTC CCCTACATCG TGACCTGGGA AGCCTTGGCT  1200
GGACCGGGCG TACCTGTGGG TCTGGTCCAG GGGATGTAGC ACTGGACCCT TCGGAACCGA  1200

TTTGACCCCC CTCCCTGGGT CAAGCCCTTT GTACACCCTA AGCCTCCGCC TCCTCTTCCT  1260
AAACTGGGGG GAGGGACCCA GTTCGGGAAA CATGTGGGAT TCGGAGGCGG AGGAGAAGGA  1260

CCATCCGCCC CGTCTCTCCC CCTTGAACCT CCTCGTTCGA CCCCGCCTCG ATCCTCCCTT  1320
GGTAGGCGGG GCAGAGAGGG GGAACTTGGA GGAGCAAGCT GGGGCGGAGC TAGGAGGGAA  1320

TATCCAGCCC TCACTCCTTC TCTAGGCGCC GGCCGCTCTA GCCCATTAAT ACGACTCACT  1380
ATAGGTCGGG AGTGAGGAAG AGATCCGCGG CCGGCGAGAT CGGGTAATTA TGCTGAGTGA  1380

ATAGGGCGAT TCGAACACCA TGCACCATCA TCATCATCAC GTCGACTATA AAGATGAGGA  1440
TATCCCGCTA AGCTTGTGGT ACGTGGTAGT AGTAGTAGTG CAGCTGATAT TTCTACTCCT  1440

CCTCGAGATG GGCGTGATTA CGGATTCACT GGCCGTCGTG GCCCGCACCG ATCGCCCTTC  1500
GGAGCTCTAC CCGCACTAAT GCCTAAGTGA CCGGCAGCAC CGGGCGTGGC TAGCGGGAAG  1500

CCAACAGTTA CGCAGCCTGA ATGGCGAATG GCGCTTTGCC TGGTTTCCGG CACCAGAAGC  1560
GGTTGTCAAT GCGTCGGACT TACCGCTTAC CGCGAAACGG ACCAAAGGCC GTGGTCTTCG  1560
```

FIG.11B-2

pICAST ALN

```
GGTGCCGGAA AGCTGGCTGG AGTGCGATCT TCCTGAGGCC GATACTGTCG TCGTCCCCTC  1620
CCACGGCCTT TCGACCGACC TCACGCTAGA AGGACTCCGG CTATGACAGC AGCAGGGGAG  1620

AAACTGGCAG ATGCACGGTT ACGATGCGCC CATCTACACC AACGTGACCT ATCCCATTAC  1680
TTTGACCGTC TACGTGCCAA TGCTACGCGG GTAGATGTGG TTGCACTGGA TAGGGTAATG  1680

GGTCAATCCG CCGTTTGTTC CCACGGAGAA TCCGACGGGT TGTTACTCGC TCACATTTAA  1740
CCAGTTAGGC GGCAAACAAG GGTGCCTCTT AGGCTGCCCA ACAATGAGCG AGTGTAAATT  1740

TGTTGATGAA AGCTGGCTAC AGGAAGGCCA GACGCGAATT ATTTTTGATG GCGTTAACTC  1800
ACAACTACTT TCGACCGATG TCCTTCCGGT CTGCGCTTAA TAAAAACTAC CGCAATTGAG  1800

GGCGTTTCAT CTGTGGTGCA ACGGGCGCTG GGTCGGTTAC GGCCAGGACA GTCGTTTGCC  1860
CCGCAAAGTA GACACCACGT TGCCCGCGAC CCAGCCAATG CCGGTCCTGT CAGCAAACGG  1860

GTCTGAATTT GACCTGAGCG CATTTTTACG CGCCGGAGAA AACCGCCTCG CGGTGATGGT  1920
CAGACTTAAA CTGGACTCGC GTAAAAATGC GCGGCCTCTT TTGGCGGAGC GCCACTACCA  1920

GCTGGGCTGG AGTGACGGCA GTTATCTGGA AGATCAGGAT ATGTGGCGGA TGAGCGGCAT  1980
CGACGCGACC TCACTGCCGT CAATAGACCT TCTAGTCCTA TACACCGCCT ACTCGCCGTA  1980

TTTCCGTGAC GTCTCGTTGC TGCATAAACC GACTACACAA ATCAGCGATT TCCATGTTGC  2040
AAAGGCACTG CAGAGCAACG ACGTATTTGG CTGATGTGTT TAGTCGCTAA AGGTACAACG  2040

CACTCGCTTT AATGATGATT RCAGCCGCGC TGTACTGGAG GCTGAAGTTC AGATGTGCGG  2100
GTGAGCGAAA TTACTACTAA AGTCGGCGCG ACATGACCTC CGACTTCAAG TCTACACGCC  2100

CGAGTTGCGT GACTACCTAC GGGTAACAGT TTCTTTATGG CAGGGTGAAA CGCAGGTCGC  2160
GCTCAACGCA CTGATGGATG CCCATTGTCA AAGAAATACC GTCCCACTTT GCGTCCAGCG  2160

CAGCGGCACC GCGCCTTTCG GCGGTGAAAT TATCGATGAG CGTGGTGGTT ATGCCGATCG  2220
GTCGCCGTGG CGCGGAAAGC CGCCACTTTA ATAGCTACTC GCACCACCAA TACGGCTAGC  2220

CGTCACACTA CGTCTGAACG TCGAAAACCC GAAACTGTGG AGCGCCGAAA TCCCGAATCT  2280
GCAGTGTGAT GCAGACTTGC AGCTTTTGGG CTTTGACACC TCGCGGCTTT AGGGCTTAGA  2280

CTATCGTGCG GTGGTTGAAC TGCACACCGC CGACGGCACG CTGATTGAAG CAGAAGCCTG  2340
GATAGCACGC CACCAACTTG ACGTGTGGCG GCTGCCGTGC GACTAACTTC GTCTTCGGAC  2340
```

FIG.11B-3

pICAST ALN

```
CGATGTCGGT TTCCGCGAGG TGCGGATTGA AAATGGTCTG CTGCTGCTGA ACGGCAAGCC  2400
GCTACAGCCA AAGGCGCTCC ACGCCTAACT TTTACCAGAC GACGACGACT TGCCGTTCGG  2400

GTTGCTGATT CGAGGCGTTA ACCGTCACGA GCATCATCCT CTGCATGGTC AGGTCATGGA  2460
CAACGACTAA GCTCCGCAAT TGGCAGTGCT CGTAGTAGGA GACGTACCAG TCCAGTACCT  2460

TGAGCAGACG ATGGTGCAGG ATATCCTGCT GATGAAGCAG AACAACTTTA ACGCCGTGCG  2520
ACTCGTCTGC TACCACGTCC TATAGGACGA CTACTTCGTC TTGTTGAAAT TGCGGCACGC  2520

CTGTTCGCAT TATCCGAACC ATCCGCTGTG GTACACGCTG TGCGACCGCT ACGGCCTGTA  2580
GACAAGCGTA ATAGGCTTGG TAGGCGACAC CATGTGCGAC ACGCTGGCGA TGCCGGACAT  2580

TGTGGTGGAT GAAGCCAATA TTGAAACCCA CGGCATGGTG CCAATGAATC GTCTGACCGA  2640
ACACCACCTA CTTCGGTTAT AACTTTGGGT GCCGTACCAC GGTTACTTAG CAGACTGGCT  2640

TGATCCGCGC TGGCTACCGG CGATGAGCGA ACGCGTAACG CGAATGGTGC AGCGCGATCG  2700
ACTAGGCGCG ACCGATGGCC GCTACTCGCT TGCGCATTGC GCTTACCACG TCGCGCTAGC  2700

TAATCACCCG AGTGTGATCA TCTGGTCGCT GGGGAATGAA TCAGGCCACG GCGCTAATCA  2760
ATTAGTGGGC TCACACTAGT AGACCAGCGA CCCCTTACTT AGTCCGGTGC CGCGATTAGT  2760

CGACGCGCTG TATCGCTGGA TCAAATCTGT CGATCCTTCC CGCCCGGTGC AGTATGAAGG  2820
GCTGCGCGAC ATAGCGACCT AGTTTAGACA GCTAGGAAGG GCGGGCCACG TCATACTTCC  2820

CGGCGGAGCC GACACCACGG CCACCGATAT TATTTGCCCG ATGTACGCGC GCGTGGATGA  2880
GCCGCCTCGG CTGTGGTGCC GGTGGCTATA ATAAACGGGC TACATGCGCG CGCACCTACT  2880

AGACCAGCCC TTCCCGGCTG TGCCGAAATG GTCCATCAAA AAATGGCTTT CGCTACCTGG  2940
TCTGGTCGGG AAGGGCCGAC ACGGCTTTAC CAGGTAGTTT TTTACCGAAA GCGATGGACC  2940

AGAGACGCGC CCGCTGATCC TTTGCGAATA CGCCCACGCG ATGGGTAACA GTCTTGGCGG  3000
TCTCTGCGCG GGCGACTAGG AAACGCTTAT GCGGGTGCGC TACCCATTGT CAGAACCGCC  3000

TTTCGCTAAA TACTGGCAGG CGTTTCGTCA GTATCCCCGT TTACAGGGCG GCTTCGTCTG  3060
AAAGCGATTT ATGACCGTCC GCAAAGCAGT CATAGGGGCA AATGTCCCGC CGAAGCAGAC  3060

GGACTGGGTG GATCAGTCGC TGATTAAATA TGATGAAAAC GGCAACCCGT GGTCGGCTTA  3120
CCTGACCCAC CTAGTCAGCG ACTAATTTAT ACTACTTTTG CCGTTGGGCA CCAGCCGAAT  3120
```

FIG.11B-4

pICAST ALN

CGGCGGTGAT TTTGGCGATA CGCCGAACGA TCGCCAGTTC TGTATGAACG GTCTGGTCTT 3180
GCCGCCACTA AAACCGCTAT GCGGCTTGCT AGCGGTCAAG ACATACTTGC CAGACCAGAA 3180

TGCCGACCGC ACGCCGCATC CAGCGCTGAC GGAAGCAAAA CACCAGCAGC AGTTTTTCCA 3240
ACGGCTGGCG TGCGGCGTAG GTCGCGACTG CCTTCGTTTT GTGGTCGTCG TCAAAAAGGT 3240

GTTCCGTTTA TCCGGGCAAA CCATCGAAGT GACCAGCGAA TACCTGTTCC GTCATAGCGA 3300
CAAGGCAAAT AGGCCCGTTT GGTAGCTTCA CTGGTCGCTT ATGGACAAGG CAGTATCGCT 3300

TAACGAGCTC CTGCACTGGA TGGTGGCGCT GGATGGTAAG CCGCTGGCAA GCGGTGAAGT 3360
ATTGCTCGAG GACGTGACCT ACCACCGCGA CCTACCATTC GGCGACCGTT CGCCACTTCA 3360

GCCTCTGGAT GTCGCTCCAC AAGGTAAACA GTTGATTGAA CTGCCTGAAC TACCGCAGCC 3420
CGGAGACCTA CAGCGAGGTG TTCCATTTGT CAACTAACTT GACGGACTTG ATGGCGTCGG 3420

GGAGAGCGCC GGGCAACTCT GGCTCACAGT ACGCGTAGTG CAACCGAACG CGACCGCATG 3480
CCTCTCGCGG CCCGTTGAGA CCGAGTGTCA TGCGCATCAC GTTGGCTTGC GCTGGCGTAC 3480

GTCAGAAGCC GGGCACATCA GCGCCTGGCA GCAGTGGCGT CTGGCGGAAA ACCTCAGTGT 3540
CAGTCTTCGG CCCGTGTAGT CGCGGACCGT CGTCACCGCA GACCGCCTTT TGGAGTCACA 3540

GACGCTCCCC GCCGCGTCCC ACGCCATCCC GCATCTGACC ACCAGCGAAA TGGATTTTTG 3600
CTGCGAGGGG CGGCGCAGGG TGCGGTAGGG CGTAGACTGG TGGTCGCTTT ACCTAAAAAC 3600

CATCGAGCTG GGTAATAAGC GTTGGCAATT TAACCGCCAG TCAGGCTTTC TTTCACAGAT 3660
GTAGCTCGAC CCATTATTCG CAACCGTTAA ATTGGCGGTC AGTCCGAAAG AAAGTGTCTA 3660

GTGGATTGGC GATAAAAAAC AACTGCTGAC GCCGCTGCGC GATCAGTTCA CCCGTGCACC 3720
CACCTAACCG CTATTTTTTG TTGACGACTG CGGCGACGCG CTAGTCAAGT GGGCACGTGG 3720

GCTGGATAAC GACATTGGCG TAAGTGAAGC GACCCGCATT GACCCTAACG CCTGGGTCGA 3780
CGACCTATTG CTGTAACCGC ATTCACTTCG CTGGGCGTAA CTGGGATTGC GGACCCAGCT 3780

ACGCTGGAAG GCGGCGGGCC ATTACCAGGC CGAAGCAGCG TTGTTGCAGT GCACGGCAGA 3840
TGCGACCTTC CGCCGCCCGG TAATGGTCCG GCTTCGTCGC AACAACGTCA CGTGCCGTCT 3840

TACACTTGCT GATGCGGTGC TGATTACGAC CGCTCACGCG TGGCAGCATC AGGGGAAAAC 3900
ATGTGAACGA CTACGCCACG ACTAATGCTG GCGAGTGCGC ACCGTCGTAG TCCCCTTTTG 3900

FIG.11B-5

pICAST ALN

```
CTTATTTATC AGCCGGAAAA CCTACCGGAT TGATGGTAGT GGTCAAATGG CGATTACCGT   3960
GAATAAATAG TCGGCCTTTT GGATGGCCTA ACTACCATCA CCAGTTTACC GCTAATGGCA   3960

TGATGTTGAA GTGGCGAGCG ATACACCGCA TCCGGCGCGG ATTGGCCTGA ACTGCCAGCT   4020
ACTACAACTT CACCGCTCGC TATGTGGCGT AGGCCGCGCC TAACCGGACT TGACGGTCGA   4020

GGCGCAGGTA GCAGAGCGGG TAAACTGGCT CGGATTAGGG CCGCAAGAAA ACTATCCCGA   4080
CCGCGTCCAT CGTCTCGCCC ATTTGACCGA GCCTAATCCC GGCGTTCTTT TGATAGGGCT   4080

CCGCCTTACT GCCGCCTGTT TTGACCGCTG GGATCTGCCA TTGTCAGACA TGTATACCCC   4140
GGCGGAATGA CGGCGGACAA AACTGGCGAC CCTAGACGGT AACAGTCTGT ACATATGGGG   4140

GTACGTCTTC CCGAGCGAAA ACGGTCTGCG CTGCGGGACG CGCGAATTGA ATTATGGCCC   4200
CATGCAGAAG GGCTCGCTTT TGCCAGACGC GACGCCCTGC GCGCTTAACT TAATACCGGG   4200

ACACCAGTGG CGCGGCGACT TCCAGTTCAA CATCAGCCGC TACAGTCAAC AGCAACTGAT   4260
TGTGGTCACC GCGCCGCTGA AGGTCAAGTT GTAGTCGGCG ATGTCAGTTG TCGTTGACTA   4260

GGAAACCAGC CATCGCCATC TGCTGCACGC GGAAGAAGGC ACATGGCTGA ATATCGACGG   4320
CCTTTGGTCG GTAGCGGTAG ACGACGTGCG CCTTCTTCCG TGTACCGACT TATAGCTGCC   4320

TTTCCATATG GGGATTGGTG GCGACGACTC CTGGAGCCCG TCAGTATCGG CGGAATTCCA   4380
AAAGGTATAC CCCTAACCAC CGCTGCTGAG GACCTCGGGC AGTCATAGCC GCCTTAAGGT   4380

GCTGAGCGCC GGTCGCTACC ATTACCAGTT GGTCTGGTGT CAAAAAAGAT CTGGAGGTGG   4440
CGACTCGCGG CCAGCGATGG TAATGGTCAA CCAGACCACA GTTTTTTCTA GACCTCCACC   4440

TGGCAGCAGG CCTTGGCGCG CCGGATCCTT AATTAACAAT TGACCGGTAA TAATAGGTAG   4500
ACCGTCGTCC GGAACCGCGC GGCCTAGGAA TTAATTGTTA ACTGGCCATT ATTATCCATC   4500

ATAAGTGACT GATTAGATGC ATTGATCCCT CGACCAATTC CGGTTATTTT CCACCATATT   4560
TATTCACTGA CTAATCTACG TAACTAGGGA GCTGGTTAAG GCCAATAAAA GGTGGTATAA   4560

GCCGTCTTTT GGCAATGTGA GGGCCCGGAA ACCTGGCCCT GTCTTCTTGA CGAGCATTCC   4620
CGGCAGAAAA CCGTTACACT CCCGGGCCTT TGGACCGGGA CAGAAGAACT GCTCGTAAGG   4620

TAGGGGTCTT TCCCCTCTCG CCAAAGGAAT GCAAGGTCTG TTGAATGTCG TGAAGGAAGC   4680
ATCCCCAGAA AGGGGAGAGC GGTTTCCTTA CGTTCCAGAC AACTTACAGC ACTTCCTTCG   4680
```

FIG.11B-6

pICAST ALN

```
AGTTCCTCTG GAAGCTTCTT GAAGACAAAC AACGTCTGTA GCGACCCTTT GCAGGCAGCG  4740
TCAAGGAGAC CTTCGAAGAA CTTCTGTTTG TTGCAGACAT CGCTGGGAAA CGTCCGTCGC  4740

GAACCCCCCA CCTGGCGACA GGTGCCTCTG CGGCCAAAAG CCACGTGTAT AAGATACACC  4800
CTTGGGGGGT GGACCGCTGT CCACGGAGAC GCCGGTTTTC GGTGCACATA TTCTATGTGG  4800

TGCAAAGGCG GCACAACCCC AGTGCCACGT TGTGAGTTGG ATAGTTGTGG AAAGAGTCAA  4860
ACGTTTCCGC CGTGTTGGGG TCACGGTGCA ACACTCAACC TATCAACACC TTTCTCAGTT  4860

ATGGCTCTCC TCAAGCGTAT TCAACAAGGG GCTGAAGGAT GCCCAGAAGG TACCCCATTG  4920
TACCGAGAGG AGTTCGCATA AGTTGTTCCC CGACTTCCTA CGGGTCTTCC ATGGGGTAAC  4920

TATGGGATCT GATCTGGGGC CTCGGTGCAC ATGCTTTACA TGTGTTTAGT CGAGGTTAAA  4980
ATACCCTAGA CTAGACCCCG GAGCCACGTG TACGAAATGT ACACAAATCA GCTCCAATTT  4980

AAACGTCTAG GCCCCCCGAA CCACGGGGAC GTGGTTTTCC TTTGAAAAAC ACGATGATAA  5040
TTTGCAGATC CGGGGGGCTT GGTGCCCCTG CACCAAAAGG AAACTTTTTG TGCTACTATT  5040

TACCATGATT GAACAAGATG GATTGCACGC AGGTTCTCCG GCCGCTTGGG TGGAGAGGCT  5100
ATGGTACTAA CTTGTTCTAC CTAACGTGCG TCCAAGAGGC CGGCGAACCC ACCTCTCCGA  5100

ATTCGGCTAT GACTGGGCAC AACAGACAAT CGGCTGCTCT GATGCCGCCG TGTTCCGGCT  5160
TAAGCCGATA CTGACCCGTG TTGTCTGTTA GCCGACGAGA CTACGGCGGC ACAAGGCCGA  5160

GTCAGCGCAG GGGCGCCCGG TTCTTTTTGT CAAGACCGAC CTGTCCGGTG CCCTGAATGA  5220
CAGTCGCGTC CCCGCGGGCC AAGAAAAACA GTTCTGGCTG GACAGGCCAC GGGACTTACT  5220

ACTGCAGGAC GAGGCAGCGC GGCTATCGTG GCTGGCCACG ACGGGCGTTC CTTGCGCAGC  5280
TGACGTCCTG CTCCGTCGCG CCGATAGCAC CGACCGGTGC TGCCCGCAAG GAACGCGTCG  5280

TGTGCTCGAC GTTGTCACTG AAGCGGGAAG GGACTGGCTG CTATTGGGCG AAGTGCCGGG  5340
ACACGAGCTG CAACAGTGAC TTCGCCCTTC CCTGACCGAC GATAACCCGC TTCACGGCCC  5340

GCAGGATCTC CTGTCATCTC ACCTTGCTCC TGCCGAGAAA GTATCCATCA TGGCTGATGC  5400
CGTCCTAGAG GACAGTAGAG TGGAACGAGG ACGGCTCTTT CATAGGTAGT ACCGACTACG  5400

AATGCGGCGG CTGCATACGC TTGATCCGGC TACCTGCCCA TTCGACCACC AAGCGAAACA  5460
TTACGCCGCC GACGTATGCG AACTAGGCCG ATGGACGGGT AAGCTGGTGG TTCGCTTTGT  5460
```

FIG.11B-7

pICAST ALN

```
TCGCATCGAG CGAGCACGTA CTCGGATGGA AGCCGGTCTT GTCGATCAGG ATGATCTGGA   5520
AGCGTAGCTC GCTCGTGCAT GAGCCTACCT TCGGCCAGAA CAGCTAGTCC TACTAGACCT   5520

CGAAGAGCAT CAGGGGCTCG CGCCAGCCGA ACTGTTCGCC AGGCTCAAGG CGCGCATGCC   5580
GCTTCTCGTA GTCCCCGAGC GCGGTCGGCT TGACAAGCGG TCCGAGTTCC GCGCGTACGG   5580

CGACGGCGAG GATCTCGTCG TGACCCATGG CGATGCCTGC TTGCCGAATA TCATGGTGGA   5640
GCTGCCGCTC CTAGAGCAGC ACTGGGTACC GCTACGGACG AACGGCTTAT AGTACCACCT   5640

AAATGGCCGC TTTTCTGGAT TCATCGACTG TGGCCGGCTG GGTGTGGCGG ACCGCTATCA   5700
TTTACCGGCG AAAAGACCTA AGTAGCTGAC ACCGGCCGAC CCACACCGCC TGGCGATAGT   5700

GGACATAGCG TTGGCTACCC GTGATATTGC TGAAGAGCTT GGCGGCGAAT GGGCTGACCG   5760
CCTGTATCGC AACCGATGGG CACTATAACG ACTTCTCGAA CCGCCGCTTA CCCGACTGGC   5760

CTTCCTCGTG CTTTACGGTA TCGCCGCTCC CGATTCGCAG CGCATCGCCT TCTATCGCCT   5820
GAAGGAGCAC GAAATGCCAT AGCGGCGAGG GCTAAGCGTC GCGTAGCGGA AGATAGCGGA   5820

TCTTGACGAG TTCTTCTGAG CGGGACTCTG GGGTTCGCAT CGATAAAATA AAAGATTTTA   5880
AGAACTGCTC AAGAAGACTC GCCCTGAGAC CCCAAGCGTA GCTATTTTAT TTTCTAAAAT   5880

TTTAGTCTCC AGAAAAAGGG GGGAATGAAA GACCCCACCT GTAGGTTTGG CAAGCTAGCT   5940
AAATCAGAGG TCTTTTTCCC CCCTTACTTT CTGGGGTGGA CATCCAAACC GTTCGATCGA   5940

TAAGTAACGC CATTTTGCAA GGCATGGAAA AATACATAAC TGAGAATAGA GAAGTTCAGA   6000
ATTCATTGCG GTAAAACGTT CCGTACCTTT TTATGTATTG ACTCTTATCT CTTCAAGTCT   6000

TCAAGGTCAG GAACAGATGG AACAGCTGAA TATGGGCCAA ACAGGATATC TGTGGTAAGC   6060
AGTTCCAGTC CTTGTCTACC TTGTCGACTT ATACCCGGTT TGTCCTATAG ACACCATTCG   6060

AGTTCCTGCC CCGGCTCAGG GCCAAGAACA GATGGAACAG CTGAATATGG GCCAAACAGG   6120
TCAAGGACGG GGCCGAGTCC CGGTTCTTGT CTACCTTGTC GACTTATACC CGGTTTGTCC   6120

ATATCTGTGG TAAGCAGTTC CTGCCCCGGC TCAGGGCCAA GAACAGATGG TCCCCAGATG   6180
TATAGACACC ATTCGTCAAG GACGGGGCCG AGTCCCGGTT CTTGTCTACC AGGGGTCTAC   6180

CGGTCCAGCC CTCAGCAGTT TCTAGAGAAC CATCAGATGT TTCCAGGGTG CCCCAAGGAC   6240
GCCAGGTCGG GAGTCGTCAA AGATCTCTTG GTAGTCTACA AAGGTCCCAC GGGGTTCCTG   6240
```

FIG.11B-8

pICAST ALN

```
CTGAAATGAC CCTGTGCCTT ATTTGAACTA ACCAATCAGT TCGCTTCTCG CTTCTGTTCG  6300
GACTTTACTG GGACACGGAA TAAACTTGAT TGGTTAGTCA AGCGAAGAGC GAAGACAAGC  6300

CGCGCTTCTG CTCCCCGAGC TCAATAAAAG AGCCCACAAC CCCTCACTCG GGGCGCCAGT  6360
GCGCGAAGAC GAGGGGCTCG AGTTATTTTC TCGGGTGTTG GGGAGTGAGC CCCGCGGTCA  6360

CCTCCGATTG ACTGAGTCGC CCGGGTACCC GTGTATCCAA TAAACCCTCT TGCAGTTGCA  6420
GGAGGCTAAC TGACTCAGCG GGCCCATGGG CACATAGGTT ATTTGGGAGA ACGTCAACGT  6420

TCCGACTTGT GGTCTCGCTG TTCCTTGGGA GGGTCTCCTC TGAGTGATTG ACTACCCGTC  6480
AGGCTGAACA CCAGAGCGAC AAGGAACCCT CCCAGAGGAG ACTCACTAAC TGATGGGCAG  6480

AGCGGGGGTC TTTCATTCAT GCAGCATGTA TCAAAATTAA TTTGGTTTTT TTTCTTAAGT  6540
TCGCCCCCAG AAAGTAAGTA CGTCGTACAT AGTTTTAATT AAACCAAAAA AAAGAATTCA  6540

ATTTACATTA AATGGCCATA GTTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT  6600
TAAATGTAAT TTACCGGTAT CAACGTAATT ACTTAGCCGG TTGCGCGCCC CTCTCCGCCA  6600

AACGCATAAC CGCGAGAAGG CGAAGGAGCG AGTGACTGAG CGACGCGAGC CAGCAAGCCG  6660
TTGCGTATTG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG GTCGTTCGGC  6660

TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG  6720
ACGCCGCTCG CCATAGTCGA GTGAGTTTCC GCCATTATGC CAATAGGTGT CTTAGTCCCC  6720

ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG  6780
TATTGCGTCC TTTCTTGTAC ACTCGTTTTC CGGTCGTTTT CCGGTCCTTG GCATTTTTCC  6780

CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC  6840
GGCGCAACGA CCGCAAAAAG GTATCCGAGG CGGGGGGACT GCTCGTAGTG TTTTTAGCTG  6840

GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG  6900
CGAGTTCAGT CTCCACCGCT TTGGGCTGTC CTGATATTTC TATGGTCCGC AAAGGGGGAC  6900

GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT  6960
CTTCGAGGGA GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG GACAGGCGGA  6960

TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG  7020
AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC GACATCCATA GAGTCAAGCC  7020
```

FIG.11B-9

pICAST ALN

TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT 7080
ACATCCAGCA AGCGAGGTTC GACCCGACAC ACGTGCTTGG GGGGCAAGTC GGGCTGGCGA 7080

GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC 7140
CGCGGAATAG GCCATTGATA GCAGAACTCA GGTTGGGCCA TTCTGTGCTG AATAGCGGTG 7140

TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT 7200
ACCGTCGTCG GTGACCATTG TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA 7200

TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC 7260
AGAACTTCAC CACCGGATTG ATGCCGATGT GATCTTCTTG TCATAAACCA TAGACGCGAG 7260

TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA 7320
ACGACTTCGG TCAATGGAAG CCTTTTTCTC AACCATCGAG AACTAGGCCG TTTGTTTGGT 7320

CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT 7380
GGCGACCATC GCCACCAAAA AAACAAACGT TCGTCGTCTA ATGCGCGTCT TTTTTTCCTA 7380

CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC 7440
GAGTTCTTCT AGGAAACTAG AAAAGATGCC CCAGACTGCG AGTCACCTTG CTTTTGAGTG 7440

GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTGCGGC 7500
CAATTCCCTA AAACCAGTAC TCTAATAGTT TTTCCTAGAA GTGGATCTAG GAAAACGCCG 7500

CGCAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA ATGCTTAATC 7560
GCGTTTAGTT AGATTTCATA TATACTCATT TGAACCAGAC TGTCAATGGT TACGAATTAG 7560

AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCGTTCAT CCATAGTTGC CTGACTCCCC 7620
TCACTCCGTG GATAGAGTCG CTAGACAGAT AAAGCAAGTA GGTATCAACG GACTGAGGGG 7620

GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA 7680
CAGCACATCT ATTGATGCTA TGCCCTCCCG AATGGTAGAC CGGGGTCACG ACGTTACTAT 7680

CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC AGCCGGAAGG 7740
GGCGCTCTGG GTGCGAGTGG CCGAGGTCTA AATAGTCGTT ATTTGGTCGG TCGGCCTTCC 7740

GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT TAATTGTTGC 7800
CGGCTCGCGT CTTCACCAGG ACGTTGAAAT AGGCGGAGGT AGGTCAGATA ATTAACAACG 7800

FIG.11B-10

pICAST ALN

```
CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT TGCCATTGCT   7860
GCCCTTCGAT CTCATTCATC AAGCGGTCAA TTATCAAACG CGTTGCAACA ACGGTAACGA   7860

ACAGGCATCG TGGTGTCACG CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA   7920
TGTCCGTAGC ACCACAGTGC GAGCAGCAAA CCATACCGAA GTAAGTCGAG GCCAAGGGTT   7920

CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT   7980
GCTAGTTCCG CTCAATGTAC TAGGGGGTAC AACACGTTTT TTCGCCAATC GAGGAAGCCA   7980

CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA   8040
GGAGGCTAGC AACAGTCTTC ATTCAACCGG CGTCACAATA GTGAGTACCA ATACCGTCGT   8040

CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC TGGTGAGTAC   8100
GACGTATTAA GAGAATGACA GTACGGTAGG CATTCTACGA AAAGACACTG ACCACTCATG   8100

TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCGACCGA GTTGCTCTTG CCCGGCGTCA   8160
AGTTGGTTCA GTAAGACTCT TATCACATAC GCCGCTGGCT CAACGAGAAC GGGCCGCAGT   8160

ATACGGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT TGGAAAACGT   8220
TATGCCCTAT TATGGCGCGG TGTATCGTCT TGAAATTTTC ACGAGTAGTA ACCTTTTGCA   8220

TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC   8280
AGAAGCCCCG CTTTTGAGAG TTCCTAGAAT GGCGACAACT CTAGGTCAAG CTACATTGGG   8280

ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC TGGGTGAGCA   8340
TGAGCACGTG GGTTGACTAG AAGTCGTAGA AAATGAAAGT GGTCGCAAAG ACCCACTCGT   8340

AAAACAGGAA GGCAAAATGC CGCAAAAAAG GGAATAAGGG CGACACGGAA ATGTTGAATA   8400
TTTTGTCCTT CCGTTTTACG GCGTTTTTTC CCTTATTCCC GCTGTGCCTT TACAACTTAT   8400

CTCATACTCT TCCTTTTTCA ATATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC   8460
GAGTATGAGA AGGAAAAAGT TATAATAACT TCGTAAATAG TCCCAATAAC AGAGTACTCG   8460

GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTC     8518
CCTATGTATA AACTTACATA AATCTTTTTA TTTGTTTATC CCCAAGGCGC GTGTAAAG     8518
```

FIG.11B-11

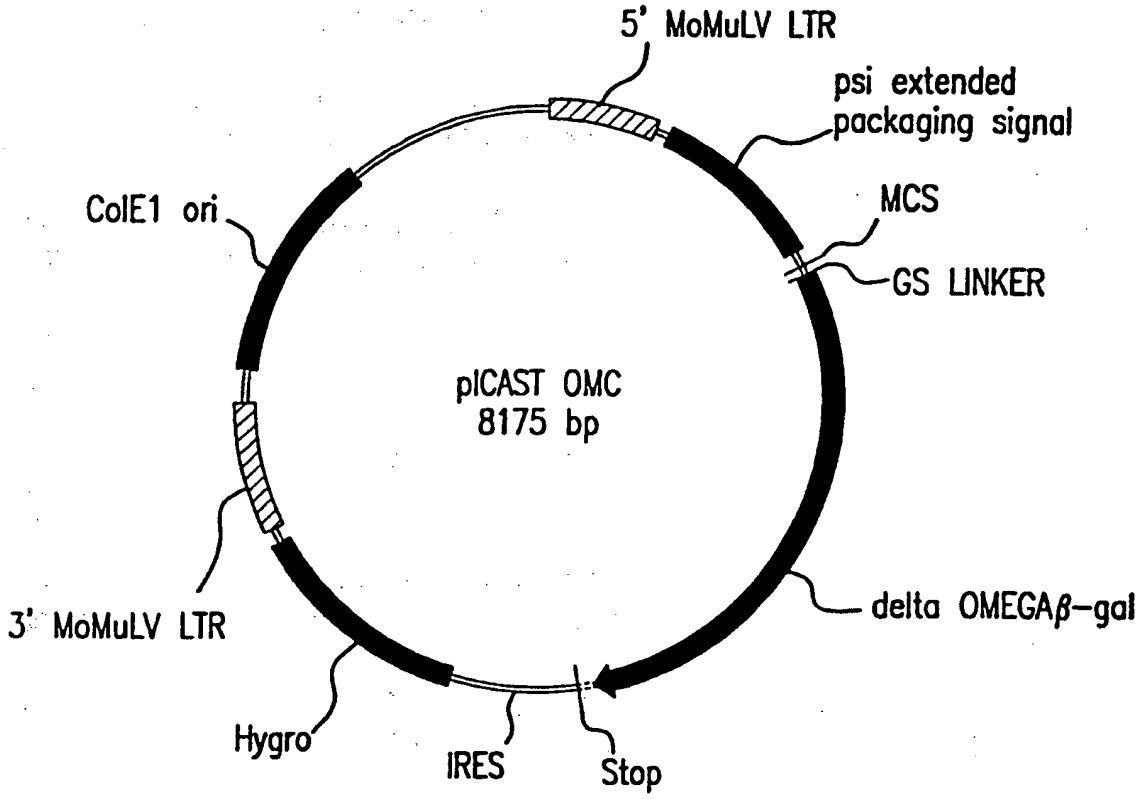
5' MoMuLV LTR
psi extended
packaging signal
MCS
GS LINKER
delta OMEGAβ–gal
Stop
IRES
Hygro
3' MoMuLV LTR
ColE1 ori
pICAST OMC
8175 bp

FIG.12A

pICAST OMC

```
CTGCAGCCTG AATATGGGCC AAACAGGATA TCTGTGGTAA GCAGTTCCTG CCCCGGCTCA    60
GACGTCGGAC TTATACCCGG TTTGTCCTAT AGACACCATT CGTCAAGGAC GGGGCCGAGT    60

GGGCCAAGAA CAGATGGAAC AGCTGAATAT GGGCCAAACA GGATATCTGT GGTAAGCAGT   120
CCCGGTTCTT GTCTACCTTG TCGACTTATA CCCGGTTTGT CCTATAGACA CCATTCGTCA   120

TCCTGCCCCG GCTCAGGGCC AAGAACAGAT GGTCCCCAGA TGCGGTCCAG CCCTCAGCAG   180
AGGACGGGGC CGAGTCCCGG TTCTTGTCTA CCAGGGGTCT ACGCCAGGTC GGGAGTCGTC   180

TTTCTAGAGA ACCATCAGAT GTTTCCAGGG TGCCCCAAGG ACCTGAAATG ACCCTGTGCC   240
AAAGATCTCT TGGTAGTCTA CAAAGGTCCC ACGGGGTTCC TGGACTTTAC TGGGACACGG   240

TTATTTGAAC TAACCAATCA GTTCGCTTCT CGCTTCTGTT CGCGCGCTTC TGCTCCCCGA   300
AATAAACTTG ATTGGTTAGT CAAGCGAAGA GCGAAGACAA GCGCGCGAAG ACGAGGGGCT   300

GCTCAATAAA AGAGCCCACA ACCCCTCACT CGGGGCGCCA GTCCTCCGAT TGACTGAGTC   360
CGAGTTATTT TCTCGGGTGT TGGGGAGTGA GCCCCGCGGT CAGGAGGCTA ACTGACTCAG   360

GCCCGGGTAC CCGTGTATCC AATAAACCCT CTTGCAGTTG CATCCGACTT GTGGTCTCGC   420
CGGGCCCATG GGCACATAGG TTATTTGGGA GAACGTCAAC GTAGGCTGAA CACCAGAGCG   420

TGTTCCTTGG GAGGYTCTCC TCTGAGTGAT TGACTACCCG TCAGCGGGGG TCTTTCATTT   480
ACAAGGAACC CTCCCAGAGG AGACTCACTA ACTGATGGGC AGTCGCCCCC AGAAAGTAAA   480

GGGGGCTCGT CCGGGATCGG GAGACCCCTG CCCAGGGACC ACCGACCCAC CACCGGGAGG   540
CCCCCGAGCA GGCCCTAGCC CTCTGGGGAC GGGTCCCTGG TGGCTGGGTG GTGGCCCTCC   540

CAAGCTGGCC AGCAACTTAT CTGTGTCTGT CCGATTGTCT AGTGTCTATG ACTGATTTTA   600
GTTCGACCGG TCGTTGAATA GACACAGACA GGCTAACAGA TCACAGATAC TGACTAAAAT   600

TGCGCCTGCG TCGGTACTAG TTAGCTAACT AGCTCTGTAT CTGGCGGACC CGTGGTGGAA   660
ACGCGGACGC AGCCATGATC AATCGATTGA TCGAGACATA GACCGCCTGG GCACCACCTT   660

CTGACGAGTT CTGAACACCC GGCCGCAACC CTGGGAGACG TCCCAGGGAC TTTGGGGGCC   720
GACTGCTCAA GACTTGTGGG CCGGCGTTGG GACCCTCTGC AGGGTCCCTG AAACCCCCGG   720

GTTTTTGTGG CCCGACCTGA GGAAGGGAGT CGATGTGGAA TCCGACCCCG TCAGGATATG   780
CAAAAACACC GGGCTGGACT CCTTCCCTCA GCTACACCTT AGGCTGGGGC AGTCCTATAC   780
```

FIG.12B-1

pICAST OMC

```
TGGTTCTGGT AGGAGACGAG AACCTAAAAC AGTTCCCGCC TCCGTCTGAA TTTTTGCTTT   840
ACCAAGACCA TCCTCTGCTC TTGGATTTTG TCAAGGGCGG AGGCAGACTT AAAAACGAAA   840

CGGTTTGGAA CCGAAGCCGC GCGTCTTGTC TGCTGCAGCA TCGTTCTGTG TTGTCTCTGT   900
GCCAAACCTT GGCTTCGGCG CGCAGAACAG ACGACGTCGT AGCAAGACAC AACAGAGACA   900

CTGACTGTGT TTCTGTATTT GTCTGAAAAT TAGGGCCAGA CTGTTACCAC TCCCTTAAGT   960
GACTGACACA AAGACATAAA CAGACTTTTA ATCCCGGTCT GACAATGGTG AGGGAATTCA   960

TTGACCTTAG GTAACTGGAA AGATGTCGAG CGGCTCGCTC ACAACCAGTC GGTAGATGTC  1020
AACTGGAATC CATTGACCTT TCTACAGCTC GCCGAGCGAG TGTTGGTCAG CCATCTACAG  1020

AAGAAGAGAC GTTGGGTTAC CTTCTGCTCT GCAGAATGGC CAACCTTTAA CGTCGGATGG  1080
TTCTTCTCTG CAACCCAATG GAAGACGAGA CGTCTTACCG GTTGGAAATT GCAGCCTACC  1080

CCGCGAGACG GCACCTTTAA CCGAGACCTC ATCACCCAGG TTAAGATCAA GGTCTTTTCA  1140
GGCGCTCTGC CGTGGAAATT GGCTCTGGAG TAGTGGGTCC AATTCTAGTT CCAGAAAAGT  1140

CCTGGCCCGC ATGGACACCC AGACCAGGTC CCCTACATCG TGACCTGGGA AGCCTTGGCT  1200
GGACCGGGCG TACCTGTGGG TCTGGTCCAG GGGATGTAGC ACTGGACCCT TCGGAACCGA  1200

TTTGACCCCC CTCCCTGGGT CAAGCCCTTT GTACACCCTA AGCCTCCGCC TCCTCTTCCT  1260
AAACTGGGGG GAGGGACCCA GTTCGGGAAA CATGTGGGAT TCGGAGGCGG AGGAGAAGGA  1260

CCATCCGCCC CGTCTCTCCC CCTTGAACCT CCTCGTTCGA CCCCGCCTCG ATCCTCCCTT  1320
GGTAGGCGGG GCAGAGAGGG GGAACTTGGA GGAGCAAGCT GGGGCGGAGC TAGGAGGGAA  1320

TATCCAGCCC TCACTCCTTC TCTAGGCGCC GGCCGCTCTA GCCCATTAAT ACGACTCACT  1380
ATAGGTCGGG AGTGAGGAAG AGATCCGCGG CCGGCGAGAT CGGGTAATTA TGCTGAGTGA  1380

ATAGGGCGAT TCGAATCAGG CCTTGGCGCG CCGGATCCTT AATTAAGCGC AATTGGGAGG  1440
TATCCCGCTA AGCTTAGTCC GGAACCGCGC GGCCTAGGAA TTAATTCGCG TTAACCCTCC  1440

TGGCGGTAGC CTCGAGATGG GCGTGATTAC GGATTCACTG GCCGTCGTTT TACAACGTCG  1500
ACCGCCATCG GAGCTCTACC CGCACTAATG CCTAAGTGAC CGGCAGCAAA ATGTTGCAGC  1500

TGACTGGGAA AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC  1560
ACTGACCCTT TTGGGACCGC AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG  1560
```

FIG.12B-2

pICAST OMC

```
CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TACGCAGCCT  1620
GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ATGCGTCGGA  1620

GAATGGCGAA TGGCGCTTTG CCTGGTTTCC GGCACCAGAA GCGGTGCCGG AAAGCTGGCT  1680
CTTACCGCTT ACCGCGAAAC GGACCAAAGG CCGTGGTCTT CGCCACGGCC TTTCGACCGA  1680

GGAGTGCGAT CTTCCTGAGG CCGATACTGT CGTCGTCCCC TCAAACTGGC AGATGCACGG  1740
CCTCACGCTA GAAGGACTCC GGCTATGACA GCAGCAGGGG AGTTTGACCG TCTACGTGCC  1740

TTACGATGCG CCCATCTACA CCAACGTGAC CTATCCCATT ACGGTCAATC CGCCGTTTGT  1800
AATGCTACGC GGGTAGATGT GGTTGCACTG GATAGGGTAA TGCCAGTTAG GCGGCAAACA  1800

TCCCACGGAG AATCCGACGG GTTGTTACTC GCTCACATTT AATGTTGATG AAAGCTGGCT  1860
AGGGTGCCTC TTAGGCTGCC CAACAATGAG CGAGTGTAAA TTACAACTAC TTTCGACCGA  1860

ACAGGAAGGC CAGACGCGAA TTATTTTTGA TGGCGTTAAC TCGGCGTTTC ATCTGTGGTG  1920
TGTCCTTCCG GTCTGCGCTT AATAAAAACT ACCGCAATTG AGCCGCAAAG TAGACACCAC  1920

CAACGGGCGC TGGGTCGGTT ACGGCCAGGA CAGTCGTTTG CCGTCTGAAT TTGACCTGAG  1980
GTTGCCCGCG ACCCAGCCAA TGCCGGTCCT GTCAGCAAAC GGCAGACTTA AACTGGACTC  1980

CGCATTTTTA CGCGCCGGAG AAAACCGCCT CGCGGTGATG GTGCTGCGCT GGAGTGACGG  2040
GCGTAAAAAT GCGCGGCCTC TTTTGGCGGA GCGCCACTAC CACGACGCGA CCTCACTGCC  2040

CAGTTATCTG GAAGATCAGG ATATGTGGCG GATGAGCGGC ATTTTCCGTG ACGTCTCGTT  2100
GTCAATAGAC CTTCTAGTCC TATACACCGC CTACTCGCCG TAAAAGGCAC TGCAGAGCAA  2100

GCTGCATAAA CCGACTACAC AAATCAGCGA TTTCCATGTT GCCACTCGCT TTAATGATGA  2160
CGACGTATTT GGCTGATGTG TTTAGTCGCT AAAGGTACAA CGGTGAGCGA AATTACTACT  2160

TTTCAGCCGC GCTGTACTGG AGGCTGAAGT TCAGATGTGC GGCGAGTTGC GTGACTACCT  2220
AAAGTCGGCG CGACATGACC TCCGACTTCA AGTCTACACG CCGCTCAACG CACTGATGGA  2220

ACGGGTAACA GTTTCTTTAT GGCAGGGTGA AACGCAGGTC GCCAGCGGCA CCGCGCCTTT  2280
TGCCCATTGT CAAAGAAATA CCGTCCCACT TTGCGTCCAG CGGTCGCCGT GGCGCGGAAA  2280

CGGCGGTGAA ATTATCGATG AGCGTGGTGG TTATGCCGAT CGCGTCACAC TACGTCTGAA  2340
GCCGCCACTT TAATAGCTAC TCGCACCACC AATACGGCTA GCGCAGTGTG ATGCAGACTT  2340
```

FIG.12B-3

pICAST OMC

CGTCGAAAAC CCGAAACTGT GGAGCGCCGA AATCCCGAAT CTCTATCGTG CGGTGGTTGA 2400
GCAGCTTTTG GGCTTTGACA CCTCGCGGCT TTAGGGCTTA GAGATAGCAC GCCACCAACT 2400

ACTGCACACC GCCGACGGCA CGCTGATTGA AGCAGAAGCC TGCGATGTCG GTTTCCGCGA 2460
TGACGTGTGG CGGCTGCCGT GCGACTAACT TCGTCTTCGG ACGCTACAGC CAAAGGCGCT 2460

GGTGCGGATT GAAAATGGTC TGCTGCTGCT GAACGGCAAG CCGTTGCTGA TTCGAGGCGT 2520
CCACGCCTAA CTTTTACCAG ACGACGACGA CTTGCCGTTC GGCAACGACT AAGCTCCGCA 2520

TAACCGTCAC GAGCATCATC CTCTGCATGG TCAGGTCATG GATGAGCAGA CGATGGTGCA 2580
ATTGGCAGTG CTCGTAGTAG GAGACGTACC AGTCCAGTAC CTACTCGTCT GCTACCACGT 2580

GGATATCCTG CTGATGAAGC AGAACAACTT TAACGCCGTG CGCTGTTCGC ATTATCCGAA 2640
CCTATAGGAC GACTACTTCG TCTTGTTGAA ATTGCGGCAC GCGACAAGCG TAATAGGCTT 2640

CCATCCGCTG TGGTACACGC TGTGCGACCG CTACGGCCTG TATGTGGTGG ATGAAGCCAA 2700
GGTAGGCGAC ACCATGTGCG ACACGCTGGC GATGCCGGAC ATACACCACC TACTTCGGTT 2700

TATTGAAACC CACGGCATGG TGCCAATGAA TCGTCTGACC GATGATCCGC GCTGGCTACC 2760
ATAACTTTGG GTGCCGTACC ACGGTTACTT AGCAGACTGG CTACTAGGCG CGACCGATGG 2760

GGCGATGAGC GAACGCGTAA CGCGAATGGT GCAGCGCGAT CGTAATCACC CGAGTGTGAT 2820
CCGCTACTCG CTTGCGCATT GCGCTTACCA CGTCGCGCTA GCATTAGTGG GCTCACACTA 2820

CATCTGGTCG CTGGGGAATG AATCAGGCCA CGGCGCTAAT CACGACGCGC TGTATCGCTG 2880
GTAGACCAGC GACCCCTTAC TTAGTCCGGT GCCGCGATTA GTGCTGCGCG ACATAGCGAC 2880

GATCAAATCT GTCGATCCTT CCCGCCCGGT GCAGTATGAA GGCGGCGGAG CCGACACCAC 2940
CTAGTTTAGA CAGCTAGGAA GGGCGGGCCA CGTCATACTT CCGCCGCCTC GGCTGTGGTG 2940

GGCCACCGAT ATTATTTGCC CGATGTACGC GCGCGTGGAT GAAGACCAGC CCTTCCCGGC 3000
CCGGTGGCTA TAATAAACGG GCTACATGCG CGCGCACCTA CTTCTGGTCG GGAAGGGCCG 3000

TGTGCCGAAA TGGTCCATCA AAAAATGGCT TTCGCTACCT GGAGAGACGC GCCCGCTGAT 3060
ACACGGCTTT ACCAGGTAGT TTTTTACCGA AAGCGATGGA CCTCTCTGCG CGGGCGACTA 3060

CCTTTGCGAA TACGCCCACG CGATGGGTAA CAGTCTTGGC GGTTTCGCTA AATACTGGCA 3120
GGAAACGCTT ATGCGGGTGC GCTACCCATT GTCAGAACCG CCAAAGCGAT TTATGACCGT 3120

FIG.12B-4

pICAST OMC

GGCGTTTCGT CAGTATCCCC GTTTACAGGG CGGCTTCGTC TGGGACTGGG TGGATCAGTC 3180
CCGCAAAGCA GTCATAGGGG CAAATGTCCC GCCGAAGCAG ACCCTGACCC ACCTAGTCAG 3180

GCTGATTAAA TATGATGAAA ACGGCAACCC GTGGTCGGCT TACGGCGGTG ATTTTGGCGA 3240
CGACTAATTT ATACTACTTT TGCCGTTGGG CACCAGCCGA ATGCCGCCAC TAAAACCGCT 3240

TACGCCGAAC GATCGCCAGT TCTGTATGAA CGGTCTGGTC TTTGCCGACC GCACGCCGCA 3300
ATGCGGCTTG CTAGCGGTCA AGACATACTT GCCAGACCAG AAACGGCTGG CGTGCGGCGT 3300

TCCAGCGCTG ACGGAAGCAA AACACCAGCA GCAGTTTTTC CAGTTCCGTT TATCCGGGCA 3360
AGGTCGCGAC TGCCTTCGTT TTGTGGTCGT CGTCAAAAAG GTCAAGGCAA ATAGGCCCGT 3360

AACCATCGAA GTGACCAGCG AATACCTGTT CCGTCATAGC GATAACGAGC TCCTGCACTG 3420
TTGGTAGCTT CACTGGTCGC TTATGGACAA GGCAGTATCG CTATTGCTCG AGGACGTGAC 3420

GATGGTGGCG CTGGATGGTA AGCCGCTGGC AAGCGGTGAA GTGCCTCTGG ATGTCGCTCC 3480
CTACCACCGC GACCTACCAT TCGGCGACCG TTCGCCACTT CACGGAGACC TACAGCGAGG 3480

ACAAGGTAAA CAGTTGATTG AACTGCCTGA ACTACCGCAG CCGGAGAGCG CCGGGCAACT 3540
TGTTCCATTT GTCAACTAAC TTGACGGACT TGATGGCGTC GGCCTCTCGC GGCCCGTTGA 3540

CTGGCTCACA GTACGCGTAG TGCAACCGAA CGCGACCGCA TGGTCAGAAG CCGGGCACAT 3600
GACCGAGTGT CATGCGCATC ACGTTGGCTT GCGCTGGCGT ACCAGTCTTC GGCCCGTGTA 3600

CAGCGCCTGG CAGCAGTGGC GTCTGGCGGA AAACCTCAGT GTGACGCTCC CCGCCGCGTC 3660
GTCGCGGACC GTCGTCACCG CAGACCGCCT TTTGGAGTCA CACTGCGAGG GGCGGCGCAG 3660

CCACGCCATC CCGCATCTGA CCACCAGCGA AATGGATTTT TGCATCGAGC TGGGTAATAA 3720
GGTGCGGTAG GGCGTAGACT GGTGGTCGCT TTACCTAAAA ACGTAGCTCG ACCCATTATT 3720

GCGTTGGCAA TTTAACCGCC AGTCAGGCTT TCTTTCACAG ATGTGGATTG GCGATAAAAA 3780
CGCAACCGTT AAATTGGCGG TCAGTCCGAA AGAAAGTGTC TACACCTAAC CGCTATTTTT 3780

ACAACTGCTG ACGCCGCTGC GCGATCAGTT CACCCGTGTC GATAGATCTG AACAGAAACT 3840
TGTTGACGAC TGCGGCGACG CGCTAGTCAA GTGGGCACAG CTATCTAGAC TTGTCTTTGA 3840

CATTTCCGAA GAAGACCTAG TCGACCATCA TCATCATCAT CACCGGTAAT AATAGGTAGA 3900
GTAAAGGCTT CTTCTGGATC AGCTGGTAGT AGTAGTAGTA GTGGCCATTA TTATCCATCT 3900

FIG.12B-5

pICAST OMC

TAAGTGACTG ATTAGATGCA TTTCGACTAG ATCCCTCGAC CAATTCCGGT TATTTTCCAC 3960
ATTCACTGAC TAATCTACGT AAAGCTGATC TAGGGAGCTG GTTAAGGCCA ATAAAAGGTG 3960

CATATTGCCG TCTTTTGGCA ATGTGAGGGC CCGGAAACCT GGCCCTGTCT TCTTGACGAG 4020
GTATAACGGC AGAAAACCGT TACACTCCCG GGCCTTTGGA CCGGGACAGA AGAACTGCTC 4020

CATTCCTAGG GGTCTTTCCC CTCTCGCCAA AGGAATGCAA GGTCTGTTGA ATGTCGTGAA 4080
GTAAGGATCC CCAGAAAGGG GAGAGCGGTT TCCTTACGTT CCAGACAACT TACAGCACTT 4080

GGAAGCAGTT CCTCTGGAAG CTTCTTGAAG ACAAACAACG TCTGTAGCGA CCCTTTGCAG 4140
CCTTCGTCAA GGAGACCTTC GAAGAACTTC TGTTTGTTGC AGACATCGCT GGGAAACGTC 4140

GCAGCGGAAC CCCCCACCTG GCGACAGGTG CCTCTGCGGC CAAAAGCCAC GTGTATAAGA 4200
CGTCGCCTTG GGGGGTGGAC CGCTGTCCAC GGAGACGCCG GTTTTCGGTG CACATATTCT 4200

TACACCTGCA AAGGCGGCAC AACCCCAGTG CCACGTTGTG AGTTGGATAG TTGTGGAAAG 4260
ATGTGGACGT TTCCGCCGTG TTGGGGTCAC GGTGCAACAC TCAACCTATC AACACCTTTC 4260

AGTCAAATGG CTCTCCTCAA GCGTATTCAA CAAGGGGCTG AAGGATGCCC AGAAGGTACC 4320
TCAGTTTACC GAGAGGAGTT CGCATAAGTT GTTCCCCGAC TTCCTACGGG TCTTCCATGG 4320

CCATTGTATG GGATCTGATC TGGGGCCTCG GTGCACATGC TTTACATGTG TTTAGTCGAG 4380
GGTAACATAC CCTAGACTAG ACCCCGGAGC CACGTGTACG AAATGTACAC AAATCAGCTC 4380

GTTAAAAAAC GTCTAGGCCC CCCGAACCAC GGGGACGTGG TTTTCCTTTG AAAAACACGA 4440
CAATTTTTTG CAGATCCGGG GGGCTTGGTG CCCCTGCACC AAAAGGAAAC TTTTTGTGCT 4440

TGATAATACC ATGAAAAAGC CTGAACTCAC CGCGACGTCT GTCGAGAAGT TTCTGATCGA 4500
ACTATTATGG TACTTTTTCG GACTTGAGTG GCGCTGCAGA CAGCTCTTCA AAGACTAGCT 4500

AAAGTTCGAC AGCGTCTCCG ACCTGATGCA GCTCTCGGAG GGCGAAGAAT CTCGTGCTTT 4560
TTTCAAGCTG TCGCAGAGGC TGGACTACGT CGAGAGCCTC CCGCTTCTTA GAGCACGAAA 4560

CAGCTTCGAT GTAGGAGGGC GTGGATATGT CCTGCGGGTA AATAGCTGCG CCGATGGTTT 4620
GTCGAAGCTA CATCCTCCCG CACCTATACA GGACGCCCAT TTATCGACGC GGCTACCAAA 4620

CTACAAAGAT CGTTATGTTT ATCGGCACTT TGCATCGGCC GCGCTCCCGA TTCCGGAAGT 4680
GATGTTTCTA GCAATACAAA TAGCCGTGAA ACGTAGCCGG CGCGAGGGCT AAGGCCTTCA 4680

FIG.12B-6

pICAST OMC

GCTTGACATT GGGGAATTTA GCGAGAGCCT GACCTATTGC ATCTCCCGCC GTGCACAGGG 4740
CGAACTGTAA CCCCTTAAAT CGCRCTCGGA CTGGATAACG TAGAGGGCGG CACGTGTCCC 4740

TGTCACGTTG CAAGACCTGC CTGAAACCGA ACTGCCCGCT GTTCTGCAGC CGGTCGCGGA 4800
ACAGTGCAAC GTTCTGGACG GACTTTGGCT TGACGGGCGA CAAGACGTCG GCCAGCGCCT 4800

GGCCATGGAT GCGATCGCTG CGGCCGATCT TAGCCAGACG AGCGGGTTCG GCCCATTCGG 4860
CCGGTACCTA CGCTAGCGAC GCCGGCTAGA ATCGGTCTGC TCGCCCAAGC CGGGTAAGCC 4860

ACCGCAAGGA ATCGGTCAAT ACACTACATG GCGTGATTTC ATATGCGCGA TTGCTGATCC 4920
TGGCGTTCCT TAGCCAGTTA TGTGATGTAC CGCACTAAAG TATACGCGCT AACGACTAGG 4920

CCATGTGTAT CACTGGCAAA CTGTGATGGA CGACACCGTC AGTGCGTCCG TCGCGCAGGC 4980
GGTACACATA GTGACCGTTT GACACTACCT GCTGTGGCAG TCACGCAGGC AGCGCGTCCG 4980

TCTCGATGAG CTGATGCTTT GGGCCGAGGA CTGCCCCGAA GTCCGGCACC TCGTGCACGC 5040
AGAGCTACTC GACTACGAAA CCCGGCTCCT GACGGGGCTT CAGGCCGTGG AGCACGTGCG 5040

GGATTTCGGC TCCAACAATG TCCTGACGGA CAATGGCCGC ATAACAGCGG TCATTGACTG 5100
CCTAAAGCCG AGGTTGTTAC AGGACTGCCT GTTACCGGCG TATTGTCGCC AGTAACTGAC 5100

GAGCGAGGCG ATGTTCGGGG ATTCCCAATA CGAGGTCGCC AACATCTTCT TCTGGAGGCC 5160
CTCGCTCCGC TACAAGCCCC TAAGGGTTAT GCTCCAGCGG TTGTAGAAGA AGACCTCCGG 5160

GTGGTTGGCT TGTATGGAGC AGCAGACGCG CTACTTCGAG CGGAGGCATC CGGAGCTTGC 5220
CACCAACCGA ACATACCTCG TCGTCTGCGC GATGAAGCTC GCCTCCGTAG GCCTCGAACG 5220

AGGATCGCCG CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 5280
TCCTAGCGGC GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGCTTG AGATAGTCTC 5280

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG ACGCAATCGT 5340
GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC TGCGTTAGCA 5340

CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG 5400
GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC 5400

GACCGATGGC TGTGTAGAAG TACTCGCCGA TAGTGGAAAC CGACGCCCCA GCACTCGTCC 5460
CTGGCTACCG ACACATCTTC ATGAGCGGCT ATCACCTTTG GCTGCGGGGT CGTGAGCAGG 5460

FIG.12B-7

pICAST OMC

GAGGGCAAAG GAATAGAGTA GATGCCGACC GGGATCTATC GATAAAATAA AAGATTTTAT 5520
CTCCCGTTTC CTTATCTCAT CTACGGCTGG CCCTAGATAG CTATTTTATT TTCTAAAATA 5520

TTAGTCTCCA GAAAAAGGGG GGAATGAAAG ACCCCACCTG TAGGTTTGGC AAGCTAGCTT 5580
AATCAGAGGT CTTTTTCCCC CCTTACTTTC TGGGGTGGAC ATCCAAACCG TTCGATCGAA 5580

AAGTAACGCC ATTTTGCAAG GCATGGAAAA ATACATAACT GAGAATAGAG AAGTTCAGAT 5640
TTCATTGCGG TAAAACGTTC CGTACCTTTT TATGTATTGA CTCTTATCTC TTCAAGTCTA 5640

CAAGGTCAGG AACAGATGGA ACAGCTGAAT ATGGGCCAAA CAGGATATCT GTGGTAAGCA 5700
GTTCCAGTCC TTGTCTACCT TGTCGACTTA TACCCGGTTT GTCCTATAGA CACCATTCGT 5700

GTTCCTGCCC CGGCTCAGGG CCAAGAACAG ATGGAACAGC TGAATATGGG CCAAACAGGA 5760
CAAGGACGGG GCCGAGTCCC GGTTCTTGTC TACCTTGTCG ACTTATACCC GGTTTGTCCT 5760

TATCTGTGGT AAGCAGTTCC TGCCCCGGCT CAGGGCCAAG AACAGATGGT CCCCAGATGC 5820
ATAGACACCA TTCGTCAAGG ACGGGGCCGA GTCCCGGTTC TTGTCTACCA GGGGTCTACG 5820

GGTCCAGCCC TCAGCAGTTT CTAGAGAACC ATCAGATGTT TCCAGGGTGC CCCAAGGACC 5880
CCAGGTCGGG AGTCGTCAAA GATCTCTTGG TAGTCTACAA AGGTCCCACG GGGTTCCTGG 5880

TGAAATGACC CTGTGCCTTA TTTGAACTAA CCAATCAGTT CGCTTCTCGC TTCTGTTCGC 5940
ACTTTACTGG GACACGGAAT AAACTTGATT GGTTAGTCAA GCGAAGAGCG AAGACAAGCG 5940

GCGCTTCTGC TCCCCGAGCT CAATAAAAGA GCCCACAACC CCTCACTCGG GGCGCCAGTC 6000
CGCGAAGACG AGGGGCTCGA GTTATTTTCT CGGGTGTTGG GGAGTGAGCC CCGCGGTCAG 6000

CTCCGATTGA CTGAGTCGCC CGGGTACCCG TGTATCCAAT AAACCCTCTT GCAGTTGCAT 6060
GAGGCTAACT GACTCAGCGG GCCCATGGGC ACATAGGTTA TTTGGGAGAA CGTCAACGTA 6060

CCGACTTGTG GTCTCGCTGT TCCTTGGGAG GGTCTCCTCT GAGTGATTGA CTACCCGTCA 6120
GGCTGAACAC CAGAGCGACA AGGAACCCTC CCAGAGGAGA CTCACTAACT GATGGGCAGT 6120

GCGGGGGTCT TTCATTCATG CAGCATGTAT CAAAATTAAT TTGGTTTTTT TTCTTAAGTA 6180
CGCCCCCAGA AAGTAAGTAC GTCGTACATA GTTTTAATTA AACCAAAAAA AAGAATTCAT 6180

TTTACATTAA ATGGCCATAG TTGCATTAAT GAATCGGCCA ACGCGCGGGG AGAGGCGGTT 6240
AAATGTAATT TACCGGTATC AACGTAATTA CTTAGCCGGT TGCGCGCCCC TCTCCGCCAA 6240

FIG.12B-8

pICAST OMC

TGCGTATTGG CGCTCTTCCG CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT 6300
ACGCATAACC GCGAGAAGGC GAAGGAGCGA GTGACTGAGC GACGCGAGCC AGCAAGCCGA 6300

GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA 6360
CGCCGCTCGC CATAGTCGAG TGAGTTTCCG CCATTATGCC AATAGGTGTC TTAGTCCCCT 6360

TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC 6420
ATTGCGTCCT TTCTTGTACA CTCGTTTTCC GGTCGTTTTC CGGTCCTTGG CATTTTTCCG 6420

CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG 6480
GCGCAACGAC CGCAAAAAGG TATCCGAGGC GGGGGGACTG CTCGTAGTGT TTTTAGCTGC 6480

CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG 6540
GAGTTCAGTC TCCACCGCTT TGGGCTGTCC TGATATTTCT ATGGTCCGCA AAGGGGGACC 6540

AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCCTT 6600
TTCGAGGGAG CACGCGAGAG GACAAGGCTG GGACGGCGAA TGGCCTATGG ACAGGCGGAA 6600

TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT 6660
AGAGGGAAGC CCTTCGCACC GCGAAAGAGT ATCGAGTGCG ACATCCATAG AGTCAAGCCA 6660

GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG 6720
CATCCAGCAA GCGAGGTTCG ACCCGACACA CGTGCTTGGG GGGCAAGTCG GGCTGGCGAC 6720

CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT 6780
GCGGAATAGG CCATTGATAG CAGAACTCAG GTTGGGCCAT TCTGTGCTGA ATAGCGGTGA 6780

GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT 6840
CCGTCGTCGG TGACCATTGT CCTAATCGTC TCGCTCCATA CATCCGCCAC GATGTCTCAA 6840

CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGAACA GTATTTGGTA TCTGCGCTCT 6900
GAACTTCACC ACCGGATTGA TGCCGATGTG ATCTTCTTGT CATAAACCAT AGACGCGAGA 6900

GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC 6960
CGACTTCGGT CAATGGAAGC CTTTTTCTCA ACCATCGAGA ACTAGGCCGT TTGTTTGGTG 6960

CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC 7020
GCGACCATCG CCACCAAAAA AACAAACGTT CGTCGTCTAA TGCGCGTCTT TTTTTCCTAG 7020

FIG.12B-9

pICAST OMC

TCAAGAAGAT CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG 7080
AGTTCTTCTA GGAAACTAGA AAAGATGCCC CAGACTGCGA GTCACCTTGC TTTTGAGTGC 7080

TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA 7140
AATTCCCTAA AACCAGTACT CTAATAGTTT TTCCTAGAAG TGGATCTAGG AAAATTTAAT 7140

AAAATGAAGT TTGCGGCCGC AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA 7200
TTTTACTTCA AACGCCGGCG TTTAGTTAGA TTTCATATAT ACTCATTTGA ACCAGACTGT 7200

GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA 7260
CAATGGTTAC GAATTAGTCA CTCCGTGGAT AGAGTCGCTA GACAGATAAA GCAAGTAGGT 7260

TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC 7320
ATCAACGGAC TGAGGGGCAG CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG 7320

CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA 7380
GGTCACGACG TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT 7380

ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC GCCTCCATCC 7440
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG CGGAGGTAGG 7440

AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA 7500
TCAGATAATT AACAACGGCC CTTCGATCTC ATTCATCAAG CGGTCAATTA TCAAACGCGT 7500

ACGTTGTTGC CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCTTCAT 7560
TGCAACAACG GTAACGATGT CCGTAGCACC ACAGTGCGAG CAGCAAACCA TACCGAAGTA 7560

TCAGCTCCGG TTCCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG 7620
AGTCGAGGCC AAGGGTTGCT AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC 7620

CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC 7680
GCCAATCGAG GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG 7680

TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTTT 7740
AGTACCAATA CCGTCGTGAC GTATTAAGAG AATGACAGTA CGGTAGGCAT TCTACGAAAA 7740

CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT 7800
GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT CACATACGCC GCTGGCTCAA 7800

FIG.12B-10

pICAST OMC

GCTCTTGCCC GGCGTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC 7860
CGAGAACGGG CCGCAGTTAT GCCCTATTAT GGCGCGGTGT ATCGTCTTGA AATTTTCACG 7860

TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT 7920
AGTAGTAACC TTTTGCAAGA AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA 7920

CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA 7980
GGTCAAGCTA CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT 7980

GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA ATAAGGGCGA 8040
CGCAAAGACC CACTCGTTTT TGTCCTTCCG TTTTACGGCG TTTTTTCCCT TATTCCCGCT 8040

CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG 8100
GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT AATAACTTCG TAAATAGTCC 8100

GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG 8160
CAATAACAGA GTACTCGCCT ATGTATAAAC TTACATAAAT CTTTTTATTT GTTTATCCCC 8160

TTCCGCGCAC ATTTC 8175
AAGGCGCGTG TAAAG 8175

FIG.12B-11

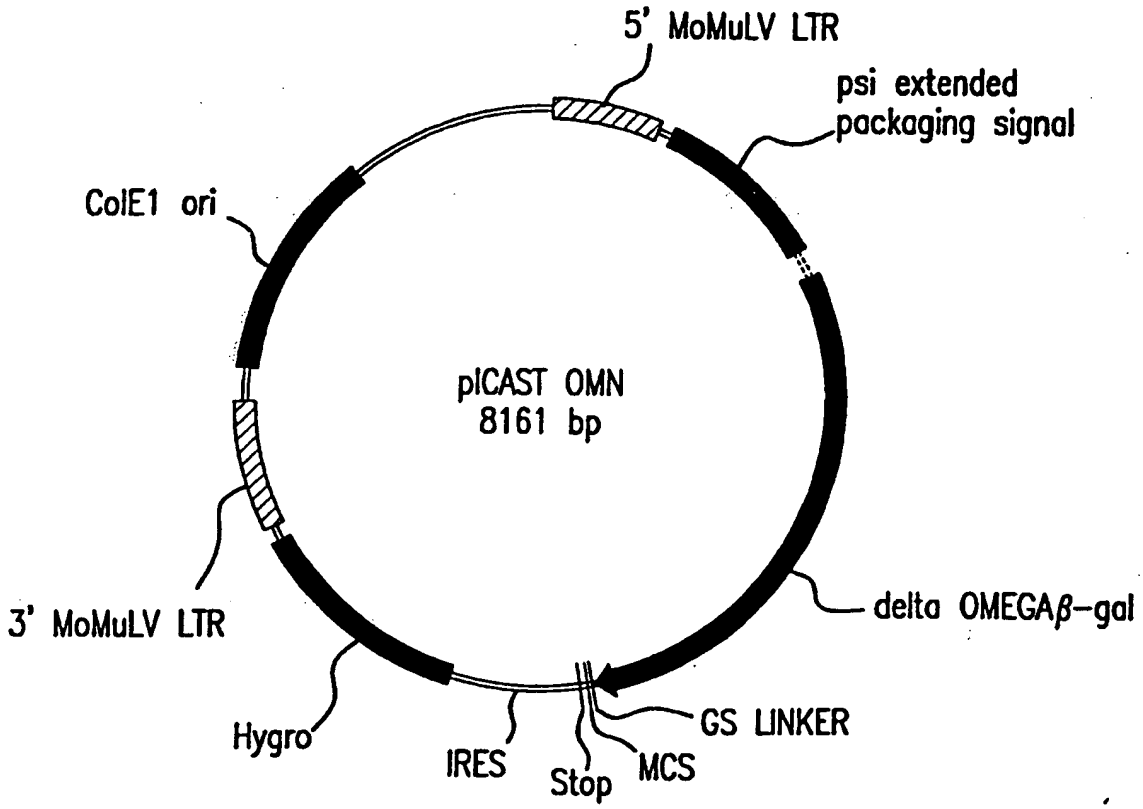
5' MoMuLV LTR
psi extended
packaging signal
ColE1 ori
pICAST OMN
8161 bp
3' MoMuLV LTR
delta OMEGAβ-gal
Hygro
IRES
Stop
MCS
GS LINKER

FIG.13A

pICAST OMN

```
CTGCAGCCTG AATATGGGCC AAACAGGATA TCTGTGGTAA GCAGTTCCTG CCCCGGCTCA    60
GACGTCGGAC TTATACCCGG TTTGTCCTAT AGACACCATT CGTCAAGGAC GGGGCCGAGT    60

GGGCCAAGAA CAGATGGAAC AGCTGAATAT GGGCCAAACA GGATATCTGT GGTAAGCAGT   120
CCCGGTTCTT GTCTACCTTG TCGACTTATA CCCGGTTTGT CCTATAGACA CCATTCGTCA   120

TCCTGCCCCG GCTCAGGGCC AAGAACAGAT GGTCCCCAGA TGCGGTCCAG CCCTCAGCAG   180
AGGACGGGGC CGAGTCCCGG TTCTTGTCTA CCAGGGGTCT ACGCCAGGTC GGGAGTCGTC   180

TTTCTAGAGA ACCATCAGAT GTTTCCAGGG TGCCCCAAGG ACCTGAAATG ACCCTGTGCC   240
AAAGATCTCT TGGTAGTCTA CAAAGGTCCC ACGGGGTTCC TGGACTTTAC TGGGACACGG   240

TTATTTGAAC TAACCAATCA GTTCGCTTCT CGCTTCTGTT CGCGCGCTTC TGCTCCCCGA   300
AATAAACTTG ATTGGTTAGT CAAGCGAAGA GCGAAGACAA GCGCGCGAAG ACGAGGGGCT   300

GCTCAATAAA AGAGCCCACA ACCCCTCACT CGGGGCGCCA GTCCTCCGAT TGACTGAGTC   360
CGAGTTATTT TCTCGGGTGT TGGGGAGTGA GCCCCGCGGT CAGGAGGCTA ACTGACTCAG   360

GCCCGGGTAC CCGTGTATCC AATAAACCCT CTTGCAGTTG CATCCGACTT GTGGTCTCGC   420
CGGGCCCATG GGCACATAGG TTATTTGGGA GAACGTCAAC GTAGGCTGAA CACCAGAGCG   420

TGTTCCTTGG GAGGGTCTCC TCTGAGTGAT TGACTACCCG TCAGCGGGGG TCTTTCATTT   480
ACAAGGAACC CTCCCAGAGG AGACTCACTA ACTGATGGGC AGTCGCCCCC AGAAAGTAAA   480

GGGGGCTCGT CCGGGATCGG GAGACCCCTG CCCAGGGACC ACCGACCCAC CACCGGGAGG   540
CCCCCGAGCA GGCCCTAGCC CTCTGGGGAC GGGTCCCTGG TGGCTGGGTG GTGGCCCTCC   540

CAAGCTGGCC AGCAACTTAT CTGTGTCTGT CCGATTGTCT AGTGTCTATG ACTGATTTTA   600
GTTCGACCGG TCGTTGAATA GACACAGACA GGCTAACAGA TCACAGATAC TGACTAAAAT   600

TGCGCCTGCG TCGGTACTAG TTAGCTAACT AGCTCTGTAT CTGGCGGACC CGTGGTGGAA   660
ACGCGGACGC AGCCATGATC AATCGATTGA TCGAGACATA GACCGCCTGG GCACCACCTT   660

CTGACGAGTT CTGAACACCC GGCCGCAACC CTGGGAGACG TCCCAGGGAC TTTGGGGGCC   720
GACTGCTCAA GACTTGTGGG CCGGCGTTGG GACCCTCTGC AGGGTCCCTG AAACCCCCGG   720

GTTTTTGTGG CCCGACCTGA GGAAGGGAGT CGATGTGGAA TCCGACCCCG TCAGGATATG   780
CAAAAACACC GGGCTGGACT CCTTCCCTCA GCTACACCTT AGGCTGGGGC AGTCCTATAC   780
```

FIG.13B-1

pICAST OMN

TGGTTCTGGT AGGAGACGAG AACCTAAAAC AGTTCCCGCC TCCGTCTGAA TTTTTGCTTT	840
ACCAAGACCA TCCTCTGCTC TTGGATTTTG TCAAGGGCGG AGGCAGACTT AAAAACGAAA	840
CGGTTTGGAA CCGAAGCCGC GCGTCTTGTC TGCTGCAGCA TCGTTCTGTG TTGTCTCTGT	900
GCCAAACCTT GGCTTCGGCG CGCAGAACAG ACGACGTCGT AGCAAGACAC AACAGAGACA	900
CTGACTGTGT TTCTGTATTT GTCTGAAAAT TAGGGCCAGA CTGTTACCAC TCCCTTAAGT	960
GACTGACACA AAGACATAAA CAGACTTTTA ATCCCGGTCT GACAATGGTG AGGGAATTCA	960
TTGACCTTAG GTAACTGGAA AGATGTCGAG CGGCTCGCTC ACAACCAGTC GGTAGATGTC	1020
AACTGGAATC CATTGACCTT TCTACAGCTC GCCGAGCGAG TGTTGGTCAG CCATCTACAG	1020
AAGAAGAGAC GTTGGGTTAC CTTCTGCTCT GCAGAATGGC CAACCTTTAA CGTCGGATGG	1080
TTCTTCTCTG CAACCCAATG GAAGACGAGA CGTCTTACCG GTTGGAAATT GCAGCCTACC	1080
CCGCGAGACG GCACCTTTAA CCGAGACCTC ATCACCCAGG TTAAGATCAA GGTCTTTTCA	1140
GGCGCTCTGC CGTGGAAATT GGCTCTGGAG TAGTGGGTCC AATTCTAGTT CCAGAAAAGT	1140
CCTGGCCCGC ATGGACACCC AGACCAGGTC CCCTACATCG TGACCTGGGA AGCCTTGGCT	1200
GGACCGGGCG TACCTGTGGG TCTGGTCCAG GGGATGTAGC ACTGGACCCT TCGGAACCGA	1200
TTTGACCCCC CTCCCTGGGT CAAGCCCTTT GTACACCCTA AGCCTCCGCC TCCTCTTCCT	1260
AAACTGGGGG GAGGGACCCA GTTCGGGAAA CATGTGGGAT TCGGAGGCGG AGGAGAAGGA	1260
CCATCCGCCC CGTCTCTCCC CCTTGAACCT CCTCGTTCGA CCCCGCCTCG ATCCTCCCTT	1320
GGTAGGCGGG GCAGAGAGGG GGAACTTGGA GGAGCAAGCT GGGGCGGAGC TAGGAGGGAA	1320
TATCCAGCCC TCACTCCTTC TCTAGGCGCC GGCCGCTCTA GCCCATTAAT ACGACTCACT	1380
ATAGGTCGGG AGTGAGGAAG AGATCCGCGG CCGGCGAGAT CGGGTAATTA TGCTGAGTGA	1380
ATAGGGCGAT TCGAACACCA TGCACCATCA TCATCATCAC GTCGACGAAC AGAAACTCAT	1440
TATCCCGCTA AGCTTGTGGT ACGTGGTAGT AGTAGTAGTG CAGCTGCTTG TCTTTGAGTA	1440
TTCCGAAGAA GACCTACTCG AGATGGGCGT GATTACGGAT TCACTGGCCG TCGTTTTACA	1500
AAGGCTTCTT CTGGATGAGC TCTACCCGCA CTAATGCCTA AGTGACCGGC AGCAAAATGT	1500
ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG CACATCCCCC	1560
TGCAGCACTG ACCCTTTTGG GACCGCAATG GGTTGAATTA GCGGAACGTC GTGTAGGGGG	1560

FIG.13B-2

pICAST OMN

TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTACG 1620
AAAGCGGTCG ACCGCATTAT CGCTTCTCCG GGCGTGGCTA GCGGGAAGGG TTGTCAATGC 1620

CAGCCTGAAT GGCGAATGGC GCTTTGCCTG GTTTCCGGCA CCAGAAGCGG TGCCGGAAAG 1680
GTCGGACTTA CCGCTTACCG CGAAACGGAC CAAAGGCCGT GGTCTTCGCC ACGGCCTTTC 1680

CTGGCTGGAG TGCGATCTTC CTGAGGCCGA TACTGTCGTC GTCCCCTCAA ACTGGCAGAT 1740
GACCGACCTC ACGCTAGAAG GACTCCGGCT ATGACAGCAG CAGGGGAGTT TGACCGTCTA 1740

GCACGGTTAC GATGCGCCCA TCTACACCAA CGTGACCTAT CCCATTACGG TCAATCCGCC 1800
CGTGCCAATG CTACGCGGGT AGATGTGGTT GCACTGGATA GGGTAATGCC AGTTAGGCGG 1800

GTTTGTTCCC ACGGAGAATC CGACGGGTTG TTACTCGCTC ACATTTAATG TTGATGAAAG 1860
CAAACAAGGG TGCCTCTTAG GCTGCCCAAC AATGAGCGAG TGTAAATTAC AACTACTTTC 1860

CTGGCTACAG GAAGGCCAGA CGCGAATTAT TTTTGATGGC GTTAACTCGG CGTTTCATCT 1920
GACCGATGTC CTTCCGGTCT GCGCTTAATA AAAACTACCG CAATTGAGCC GCAAAGTAGA 1920

GTGGTGCAAC GGGCGCTGGG TCGGTTACGG CCAGGACAGT CGTTTGCCGT CTGAATTTGA 1980
CACCACGTTG CCCGCGACCC AGCCAATGCC GGTCCTGTCA GCAAACGGCA GACTTAAACT 1980

CCTGAGCGCA TTTTTACGCG CCGGAGAAAA CCGCCTCGCG GTGATGGTGC TGCGCTGGAG 2040
GGACTCGCGT AAAAATGCGC GGCCTCTTTT GGCGGAGCGC CACTACCACG ACGCGACCTC 2040

TGACGGCAGT TATCTGGAAG ATCAGGATAT GTGGCGGATG AGCGGCATTT TCCGTGACGT 2100
ACTGCCGTCA ATAGACCTTC TAGTCCTATA CACCGCCTAC TCGCCGTAAA AGGCACTGCA 2100

CTCGTTGCTG CATAAACCGA CTACACAAAT CAGCGATTTC CATGTTGCCA CTCGCTTTAA 2160
GAGCAACGAC GTATTTGGCT GATGTGTTTA GTCGCTAAAG GTACAACGGT GAGCGAAATT 2160

TGATGATTTC AGCCGCGCTG TACTGGAGGC TGAAGTTCAG ATGTGCGGCG AGTTGCGTGA 2220
ACTACTAAAG TCGGCGCGAC ATGACCTCCG ACTTCAAGTC TACACGCCGC TCAACGCACT 2220

CTACCTACGG GTAACAGTTT CTTTATGGCA GGGTGAAACG CAGGTCGCCA GCGGCACCGC 2280
GATGGATGCC CATTGTCAAA GAAATACCGT CCCACTTTGC GTCCAGCGGT CGCCGTGGCG 2280

GCCTTTCGGC GGTGAAATTA TCGATGAGCG TGGTGGTTAT GCCGATCGCG TCACACTACG 2340
CGGAAAGCCG CCACTTTAAT AGCTACTCGC ACCACCAATA CGGCTAGCGC AGTGTGATGC 2340

FIG. 13B-3

pICAST OMN

TCTGAACGTC GAAAACCCGA AACTGTGGAG CGCCGAAATC CCGAATCTCT ATCGTGCGGT 2400
AGACTTGCAG CTTTTGGGCT TTGACACCTC GCGGCTTTAG GGCTTAGAGA TAGCACGCCA 2400

GGTTGAACTG CACACCGCCG ACGGCACGCT GATTGAAGCA GAAGCCTGCG ATGTCGGTTT 2460
CCAACTTGAC GTGTGGCGGC TGCCGTGCGA CTAACTTCGT CTTCGGACGC TACAGCCAAA 2460

CCGCGAGGTG CGGATTGAAA ATGGTCTGCT GCTGCTGAAC GGCAAGCCGT TGCTGATTCG 2520
GGCGCTCCAC GCCTAACTTT TACCAGACGA CGACGACTTG CCGTTCGGCA ACGACTAAGC 2520

AGGCGTTAAC CGTCACGAGC ATCATCCTCT GCATGGTCAG GTCATGGATG AGCAGACGAT 2580
TCCGCAATTG GCAGTGCTCG TAGTAGGAGA CGTACCAGTC CAGTACCTAC TCGTCTGCTA 2580

GGTGCAGGAT ATCCTGCTGA TGAAGCAGAA CAACTTTAAC GCCGTGCGCT GTTCGCATTA 2640
CCACGTCCTA TAGGACGACT ACTTCGTCTT GTTGAAATTG CGGCACGCGA CAAGCGTAAT 2640

TCCGAACCAT CCGCTGTGGT ACACGCTGTG CGACCGCTAC GGCCTGTATG TGGTGGATGA 2700
AGGCTTGGTA GGCGACACCA TGTGCGACAC GCTGGCGATG CCGGACATAC ACCACCTACT 2700

AGCCAATATT GAAACCCACG GCATGGTGCC AATGAATCGT CTGACCGATG ATCCGCGCTG 2760
TCGGTTATAA CTTTGGGTGC CGTACCACGG TTACTTAGCA GACTGGCTAC TAGGCGCGAC 2760

GCTACCGGCG ATGAGCGAAC GCGTAACGCG AATGGTGCAG CGCGATCGTA ATCACCCGAG 2820
CGATGGCCGC TACTCGCTTG CGCATTGCGC TTACCACGTC GCGCTAGCAT TAGTGGGCTC 2820

TGTGATCATC TGGTCGCTGG GGAATGAATC AGGCCACGGC GCTAATCACG ACGCGCTGTA 2880
ACACTAGTAG ACCAGCGACC CCTTACTTAG TCCGGTGCCG CGATTAGTGC TGCGCGACAT 2880

TCGCTGGATC AAATCTGTCG ATCCTTCCCG CCCGGTGCAG TATGAAGGCG GCGGAGCCGA 2940
AGCGACCTAG TTTAGACAGC TAGGAAGGGC GGGCCACGTC ATACTTCCGC CGCCTCGGCT 2940

CACCACGGCC ACCGATATTA TTTGCCCGAT GTACGCGCGC GTGGATGAAG ACCAGCCCTT 3000
GTGGTGCCGG TGGCTATAAT AAACGGGCTA CATGCGCGCG CACCTACTTC TGGTCGGGAA 3000

CCCGGCTGTG CCGAAATGGT CCATCAAAAA ATGGCTTTCG CTACCTGGAG AGACGCGCCC 3060
GGGCCGACAC GGCTTTACCA GGTAGTTTTT TACCGAAAGC GATGGACCTC TCTGCGCGGG 3060

GCTGATCCTT TGCGAATACG CCCACGCGAT GGGTAACAGT CTTGGCGGTT TCGCTAAATA 3120
CGACTAGGAA ACGCTTATGC GGGTGCGCTA CCCATTGTCA GAACCGCCAA AGCGATTTAT 3120

FIG.13B-4

pICAST OMN

CTGGCAGGCG TTTCGTCAGT ATCCCCGTTT ACAGGGCGGC TTCGTCTGGG ACTGGGTGGA 3180
GACCGTCCGC AAAGCAGTCA TAGGGGCAAA TGTCCCGCCG AAGCAGACCC TGACCCACCT 3180

TCAGTCGCTG ATTAAATATG ATGAAAACGG CAACCCGTGG TCGGCTTACG GCGGTGATTT 3240
AGTCAGCGAC TAATTTATAC TACTTTTGCC GTTGGGCACC AGCCGAATGC CGCCACTAAA 3240

TGGCGATACG CCGAACGATC GCCAGTTCTG TATGAACGGT CTGGTCTTTG CCGACCGCAC 3300
ACCGCTATGC GGCTTGCTAG CGGTCAAGAC ATACTTGCCA GACCAGAAAC GGCTGGCGTG 3300

GCCGCATCCA GCGCTGACGG AAGCAAAACA CCAGCAGCAG TTTTTCCAGT TCCGTTTATC 3360
CGGCGTAGGT CGCGACTGCC TTCGTTTTGT GGTCGTCGTC AAAAAGGTCA AGGCAAATAG 3360

CGGGCAAACC ATCGAAGTGA CCAGCGAATA CCTGTTCCGT CATAGCGATA ACGAGCTCCT 3420
GCCCGTTTGG TAGCTTCACT GGTCGCTTAT GGACAAGGCA GTATCGCTAT TGCTCGAGGA 3420

GCACTGGATG GTGGCGCTGG ATGGTAAGCC GCTGGCAAGC GGTGAAGTGC CTCTGGATGT 3480
CGTGACCTAC CACCGCGACC TACCATTCGG CGACCGTTCG CCACTTCACG GAGACCTACA 3480

CGCTCCACAA GGTAAACAGT TGATTGAACT GCCTGAACTA CCGCAGCCGG AGAGCGCCGG 3540
GCGAGGTGTT CCATTTGTCA ACTAACTTGA CGGACTTGAT GGCGTCGGCC TCTCGCGGCC 3540

GCAACTCTGG CTCACAGTAC GCGTAGTGCA ACCGAACGCG ACCGCATGGT CAGAAGCCGG 3600
CGTTGAGACC GAGTGTCATG CGCATCACGT TGGCTTGCGC TGGCGTACCA GTCTTCGGCC 3600

GCACATCAGC GCCTGGCAGC AGTGGCGTCT GGCGGAAAAC CTCAGTGTGA CGCTCCCCGC 3660
CGTGTAGTCG CGGACCGTCG TCACCGCAGA CCGCCTTTTG GAGTCACACT GCGAGGGGCG 3660

CGCGTCCCAC GCCATCCCGC ATCTGACCAC CAGCGAAATG GATTTTTGCA TCGAGCTGGG 3720
GCGCAGGGTG CGGTAGGGCG TAGACTGGTG GTCGCTTTAC CTAAAAACGT AGCTCGACCC 3720

TAATAAGCGT TGGCAATTTA ACCGCCAGTC AGGCTTTCTT TCACAGATGT GGATTGGCGA 3780
ATTATTCGCA ACCGTTAAAT TGGCGGTCAG TCCGAAAGAA AGTGTCTACA CCTAACCGCT 3780

TAAAAAACAA CTGCTGACGC CGCTGCGCGA TCAGTTCACC CGTGTCGATA GATCTGGAGG 3840
ATTTTTTGTT GACGACTGCG GCGACGCGCT AGTCAAGTGG GCACAGCTAT CTAGACCTCC 3840

TGGTGGCAGC AGGCCTTGGC GCGCCGGATC CTTAATTAAC AATTGACCGG TAATAATAGG 3900
ACCACCGTCG TCCGGAACCG CGCGGCCTAG GAATTAATTG TTAACTGGCC ATTATTATCC 3900

FIG.13B-5

pICAST OMN

TAGATAAGTG ACTGATTAGA TGCATTTCGA CTAGATCCCT CGACCAATTC CGGTTATTTT 3960
ATCTATTCAC TGACTAATCT ACGTAAAGCT GATCTAGGGA GCTGGTTAAG GCCAATAAAA 3960

CCACCATATT GCCGTCTTTT GGCAATGTGA GGGCCCGGAA ACCTGGCCCT GTCTTCTTGA 4020
GGTGGTATAA CGGCAGAAAA CCGTTACACT CCCGGGCCTT TGGACCGGGA CAGAAGAACT 4020

CGAGCATTCC TAGGGGTCTT TCCCCTCTCG CCAAAGGAAT GCAAGGTCTG TTGAATGTCG 4080
GCTCGTAAGG ATCCCCAGAA AGGGGAGAGC GGTTTCCTTA CGTTCCAGAC AACTTACAGC 4080

TGAAGGAAGC AGTTCCTCTG GAAGCTTCTT GAAGACAAAC AACGTCTGTA GCGACCCTTT 4140
ACTTCCTTCG TCAAGGAGAC CTTCGAAGAA CTTCTGTTTG TTGCAGACAT CGCTGGGAAA 4140

GCAGGCAGCG GAACCCCCCA CCTGGCGACA GGTGCCTCTG CGGCCAAAAG CCACGTGTAT 4200
CGTCCGTCGC CTTGGGGGGT GGACCGCTGT CCACGGAGAC GCCGGTTTTC GGTGCACATA 4200

AAGATACACC TGCAAAGGCG GCACAACCCC AGTGCCACGT TGTGAGTTGG ATAGTTGTGG 4260
TTCTATGTGG ACGTTTCCGC CGTGTTGGGG TCACGGTGCA ACACTCAACC TATCAACACC 4260

AAAGAGTCAA ATGGCTCTCC TCAAGCGTAT TCAACAAGGG GCTGAAGGAT GCCCAGAAGG 4320
TTTCTCAGTT TACCGAGAGG AGTTCGCATA AGTTGTTCCC CGACTTCCTA CGGGTCTTCC 4320

TACCCCATTG TATGGGATCT GATCTGGGGC CTCGGTGCAC ATGCTTTACA TGTGTTTAGT 4380
ATGGGGTAAC ATACCCTAGA CTAGACCCCG GAGCCACGTG TACGAAATGT ACACAAATCA 4380

CGAGGTTAAA AAACGTCTAG GCCCCCCGAA CCACGGGGAC GTGGTTTTCC TTTGAAAAAC 4440
GCTCCAATTT TTTGCAGATC CGGGGGGCTT GGTGCCCCTG CACCAAAAGG AAACTTTTTG 4440

ACGATGATAA TACCATGAAA AAGCCTGAAC TCACCGCGAC GTCTGTCGAG AAGTTTCTGA 4500
TGCTACTATT ATGGTACTTT TTCGGACTTG AGTGGCGCTG CAGACAGCTC TTCAAAGACT 4500

TCGAAAAGTT CGACAGCGTC TCCGACCTGA TGCAGCTCTC GGAGGGCGAA GAATCTCGTG 4560
AGCTTTTCAA GCTGTCGCAG AGGCTGGACT ACGTCGAGAG CCTCCCGCTT CTTAGAGCAC 4560

CTTTCAGCTT CGATGTAGGA GGGCGTGGAT ATGTCCTGCG GGTAAATAGC TGCGCCGATG 4620
GAAAGTCGAA GCTACATCCT CCCGCACCTA TACAGGACGC CCATTTATCG ACGCGGCTAC 4620

GTTTCTACAA AGATCGTTAT GTTTATCGGC ACTTTGCATC GGCCGCGCTC CCGATTCCGG 4680
CAAAGATGTT TCTAGCAATA CAAATAGCCG TGAAACGTAG CCGGCGCGAG GGCTAAGGCC 4680

FIG.13B-6

pICAST OMN

AAGTGCTTGA CATTGGGGAA TTTAGCGAGA GCCTGACCTA TTGCATCTCC CGCCGTGCAC 4740
TTCACGAACT GTAACCCCTT AAATCGCTCT CGGACTGGAT AACGTAGAGG GCGGCACGTG 4740

AGGGTGTCAC GTTGCAAGAC CTGCCTGAAA CCGAACTGCC CGCTGTTCTG CAGCCGGTCG 4800
TCCCACAGTG CAACGTTCTG GACGGACTTT GGCTTGACGG GCGACAAGAC GTCGGCCAGC 4800

CGGAGGCCAT GGATGCGATC GCTGCGGCCG ATCTTAGCCA GACGAGCGGG TTCGGCCCAT 4860
GCCTCCGGTA CCTACGCTAG CGACGCCGGC TAGAATCGGT CTGCTCGCCC AAGCCGGGTA 4860

TCGGACCGCA AGGAATCGGT CAATACACTA CATGGCGTGA TTTCATATGC GCGATTGCTG 4920
AGCCTGGCGT TCCTTAGCCA GTTATGTGAT GTACCGCACT AAAGTATACG CGCTAACGAC 4920

ATCCCCATGT GTATCACTGG CAAACTGTGA TGGACGACAC CGTCAGTGCG TCCGTCGCGC 4980
TAGGGGTACA CATAGTGACC GTTTGACACT ACCTGCTGTG GCAGTCACGC AGGCAGCGCG 4980

AGGCTCTCGA TGAGCTGATG CTTTGGGCCG AGGACTGCCC CGAAGTCCGG CACCTCGTGC 5040
TCCGAGAGCT ACTCGACTAC GAAACCCGGC TCCTGACGGG GCTTCAGGCC GTGGAGCACG 5040

ACGCGGATTT CGGCTCCAAC AATGTCCTGA CGGACAATGG CCGCATAACA GCGGTCATTG 5100
TGCGCCTAAA GCCGAGGTTG TTACAGGACT GCCTGTTACC GGCGTATTGT CGCCAGTAAC 5100

ACTGGAGCGA GGCGATGTTC GGGGATTCCC AATACGAGGT CGCCAACATC TTCTTCTGGA 5160
TGACCTCGCT CCGCTACAAG CCCCTAAGGG TTATGCTCCA GCGGTTGTAG AAGAAGACCT 5160

GGCCGTGGTT GGCTTGTATG GAGCAGCAGA CGCGCTACTT CGAGCGGAGG CATCCGGAGC 5220
CCGGCACCAA CCGAACATAC CTCGTCGTCT GCGCGATGAA GCTCGCCTCC GTAGGCCTCG 5220

TTGCAGGATC GCCGCGGCTC CGGGCGTATA TGCTCCGCAT TGGTCTTGAC CAACTCTATC 5280
AACGTCCTAG CGGCGCCGAG GCCCGCATAT ACGAGGCGTA ACCAGAACTG GTTGAGATAG 5280

AGAGCTTGGT TGACGGCAAT TTCGATGATG CAGCTTGGGC GCAGGGTCGA TGCGACGCAA 5340
TCTCGAACCA ACTGCCGTTA AAGCTACTAC GTCGAACCCG CGTCCCAGCT ACGCTGCGTT 5340

TCGTCCGATC CGGAGCCGGG ACTGTCGGGC GTACACAAAT CGCCCGCAGA AGCGCGGCCG 5400
AGCAGGCTAG GCCTCGGCCC TGACAGCCCG CATGTGTTTA GCGGGCGTCT TCGCGCCGGC 5400

TCTGGACCGA TGGCTGTGTA GAAGTACTCG CCGATAGTGG AAACCGACGC CCCAGCACTC 5460
AGACCTGGCT ACCGACACAT CTTCATGAGC GGCTATCACC TTTGGCTGCG GGGTCGTGAG 5460

FIG.13B-7

pICAST OMN

GTCCGAGGGC AAAGGAATAG AGTAGATGCC GACCGGGATC TATCGATAAA ATAAAAGATT 5520
CAGGCTCCCG TTTCCTTATC TCATCTACGG CTGGCCCTAG ATAGCTATTT TATTTTCTAA 5520

TTATTTAGTC TCCAGAAAAA GGGGGGAATG AAGACCCCAA CCTGTAGGTT TGGCAAGCTA 5580
AATAAATCAG AGGTCTTTTT CCCCCCTTAC TTTCTGGGGT GGACATCCAA ACCGTTCGAT 5580

GCTTAAGTAA CGCCATTTTG CAAGGCATGG AAAAATACAT AACTGAGAAT AGAGAAGTTC 5640
CGAATTCATT GCGGTAAAAC GTTCCGTACC TTTTTATGTA TTGACTCTTA TCTCTTCAAG 5640

AGATCAAGGT CAGGAACAGA TGGAACAGCT GAATATGGGC CAAACAGGAT ATCTGTGGTA 5700
TCTAGTTCCA GTCCTTGTCT ACCTTGTCGA CTTATACCCG GTTTGTCCTA TAGACACCAT 5700

AGCAGTTCCT GCCCCGGCTC AGGGCCAAGA ACAGATGGAA CAGCTGAATA TGGGCCAAAC 5760
TCGTCAAGGA CGGGGCCGAG TCCCGGTTCT TGTCTACCTT GTCGACTTAT ACCCGGTTTG 5760

AGGATATCTG TGGTAAGCAG TTCCTGCCCC GGCTCAGGGC CAAGAACAGA TGGTCCCCAG 5820
TCCTATAGAC ACCATTCGTC AAGGACGGGG CCGAGTCCCG GTTCTTGTCT ACCAGGGGTC 5820

ATGCGGTCCA GCCCTCAGCA GTTTCTAGAG AACCATCAGA TGTTTCCAGG GTGCCCCAAG 5880
TACGCCAGGT CGGGAGTCGT CAAAGATCTC TTGGTAGTCT ACAAAGGTCC CACGGGGTTC 5880

GACCTGAAAT GACCCTGTGC CTTATTTGAA CTAACCAATC AGTTCGCTTC TCGCTTCTGT 5940
CTGGACTTTA CTGGGACACG GAATAAACTT GATTGGTTAG TCAAGCGAAG AGCGAAGACA 5940

TCGCGCGCTT CTGCTCCCCG AGCTCAATAA AAGAGCCCAC AACCCCTCAC TCGGGGCGCC 6000
AGCGCGCGAA GACGAGGGGC TCGAGTTATT TTCTCGGGTG TTGGGGAGTG AGCCCCGCGG 6000

AGTCCTCCGA TTGACTGAGT CGCCCGGGTA CCCGTGTATC CAATAAACCC TCTTGCAGTT 6060
TCAGGAGGCT AACTGACTCA GCGGGCCCAT GGGCACATAG GTTATTTGGG AGAACGTCAA 6060

GCATCCGACT TGTGGTCTCG CTGTTCCTTG GGAGGGTCTC CTCTGAGTGA TTGACTACCC 6120
CGTAGGCTGA ACACCAGAGC GACAAGGAAC CCTCCCAGAG GAGACTCACT AACTGATGGG 6120

GTCAGCGGGG GTCTTTCATT CATGCAGCAT GTATCAAAAT TAATTTGGTT TTTTTTCTTA 6180
CAGTCGCCCC CAGAAAGTAA GTACGTCGTA CATAGTTTTA ATTAAACCAA AAAAAAGAAT 6180

AGTATTTACA TTAAATGGCC ATAGTTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC 6240
TCATAAATGT AATTTACCGG TATCAACGTA ATTACTTAGC CGGTTGCGCG CCCCTCTCCG 6240

FIG.13B-8

pICAST OMN

GGTTTGCGTA TTGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTGCGC TCGGTCGTTC 6300
CCAAACGCAT AACCGCGAGA AGGCGAAGGA GCGAGTGACT GAGCGACGCG AGCCAGCAAG 6300

GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT ACGGTTATCC ACAGAATCAG 6360
CCGACGCCGC TCGCCATAGT CGAGTGAGTT TCCGCCATTA TGCCAATAGG TGTCTTAGTC 6360

GGGATAACGC AGGAAAGAAC ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG AACCGTAAAA 6420
CCCTATTGCG TCCTTTCTTG TACACTCGTT TTCCGGTCGT TTTCCGGTCC TTGGCATTTT 6420

AGGCCGCGTT GCTGGCGTTT TTCCATAGGC TCCGCCCCCC TGACGAGCAT CACAAAAATC 6480
TCCGGCGCAA CGACCGCAAA AAGGTATCCG AGGCGGGGGG ACTGCTCGTA GTGTTTTTAG 6480

GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG GCGTTTCCCC 6540
CTGCGAGTTC AGTCTCCACC GCTTTGGGCT GTCCTGATAT TTCTATGGTC CGCAAAGGGG 6540

CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA TACCTGTCCG 6600
GACCTTCGAG GGAGCACGCG AGAGGACAAG GCTGGGACGG CGAATGGCCT ATGGACAGGC 6600

CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCATAGCTC ACGCTGTAGG TATCTCAGTT 6660
GGAAAGAGGG AAGCCCTTCG CACCGCGAAA GAGTATCGAG TGCGACATCC ATAGAGTCAA 6660

CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT GTGTGCACGA ACCCCCCGTT CAGCCCGACC 6720
GCCACATCCA GCAAGCGAGG TTCGACCCGA CACACGTGCT TGGGGGGCAA GTCGGGCTGG 6720

GCTGCGCCTT ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC 6780
CGACGCGGAA TAGGCCATTG ATAGCAGAAC TCAGGTTGGG CCATTCTGTG CTGAATAGCG 6780

CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC GGTGCTACAG 6840
GTGACCGTCG TCGGTGACCA TTGTCCTAAT CGTCTCGCTC CATACATCCG CCACGATGTC 6840

AGTTCTTGAA GTGGTGGCCT AACTACGGCT ACACTAGAAG AACAGTATTT GGTATCTGCG 6900
TCAAGAACTT CACCACCGGA TTGATGCCGA TGTGATCTTC TTGTCATAAA CCATAGACGC 6900

CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG CTCTTGATCC GGCAAACAAA 6960
GAGACGACTT CGGTCAATGG AAGCCTTTTT CTCAACCATC GAGAACTAGG CCGTTTGTTT 6960

CCACCGCTGG TAGCGGTGGT TTTTTTGTTT GCAAGCAGCA GATTACGCGC AGAAAAAAAG 7020
GGTGGCGACC ATCGCCACCA AAAAAACAAA CGTTCGTCGT CTAATGCGCG TCTTTTTTTC 7020

FIG.13B-9

pICAST OMN

GATCTCAAGA AGATCCTTTG ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG AACGAAAACT 7080
CTAGAGTTCT TCTAGGAAAC TAGAAAAGAT GCCCCAGACT GCGAGTCACC TTGCTTTTGA 7080

CACGTTAAGG GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTCACCTAG ATCCTTTTGC 7140
GTGCAATTCC CTAAAACCAG TACTCTAATA GTTTTTCCTA GAAGTGGATC TAGGAAAACG 7140

GGCCGCAAAT CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA 7200
CCGGCGTTTA GTTAGATTTC ATATATACTC ATTTGAACCA GACTGTCAAT GGTTACGAAT 7200

ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC 7260
TAGTCACTCC GTGGATAGAG TCGCTAGACA GATAAAGCAA GTAGGTATCA ACGGACTGAG 7260

CCCGTCGTGT AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG 7320
GGGCAGCACA TCTATTGATG CTATGCCCTC CCGAATGGTA GACCGGGGTC ACGACGTTAC 7320

ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA 7380
TATGGCGCTC TGGGTGCGAG TGGCCGAGGT CTAAATAGTC GTTATTTGGT CGGTCGGCCT 7380

AGGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT 7440
TCCCGGCTCG CGTCTTCACC AGGACGTTGA AATAGGCGGA GGTAGGTCAG ATAATTAACA 7440

TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT 7500
ACGGCCCTTC GATCTCATTC ATCAAGCGGT CAATTATCAA ACGCGTTGCA ACAACGGTAA 7500

GCTACAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC 7560
CGATGTCCGT AGCACCACAG TGCGAGCAGC AAACCATACC GAAGTAAGTC GAGGCCAAGG 7560

CAACGATCAA GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC 7620
GTTGCTAGTT CCGCTCAATG TACTAGGGGG TACAACACGT TTTTTCGCCA ATCGAGGAAG 7620

GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA 7680
CCAGGAGGCT AGCAACAGTC TTCATTCAAC CGGCGTCACA ATAGTGAGTA CCAATACCGT 7680

GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG 7740
CGTGACGTAT TAAGAGAATG ACAGTACGGT AGGCATTCTA CGAAAAGACA CTGACCACTC 7740

TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG 7800
ATGAGTTGGT TCAGTAAGAC TCTTATCACA TACGCCGCTG GCTCAACGAG AACGGGCCGC 7800

FIG.13B-10

TCAATACGGG ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA 7860
AGTTATGCCC TATTATGGCG CGGTGTATCG TCTTGAAATT TTCACGAGTA GTAACCTTTT 7860

CGTTCTTCGG GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA 7920
GCAAGAAGCC CCGCTTTTGA GAGTTCCTAG AATGGCGACA ACTCTAGGTC AAGCTACATT 7920

CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA 7980
GGGTGAGCAC GTGGGTTGAC TAGAAGTCGT AGAAAATGAA AGTGGTCGCA AAGACCCACT 7980

GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA 8040
CGTTTTTGTC CTTCCGTTTT ACGGCGTTTT TTCCCTTATT CCCGCTGTGC CTTTACAACT 8040

ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG 8100
TATGAGTATG AGAAGGAAAA AGTTATAATA ACTTCGTAAA TAGTCCCAAT AACAGAGTAC 8100

AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT 8160
TCGCCTATGT ATAAACTTAC ATAAATCTTT TTATTTGTTT ATCCCCAAGG CGCGTGTAAA 8160

C 8161
G 8161

FIG.13B-11

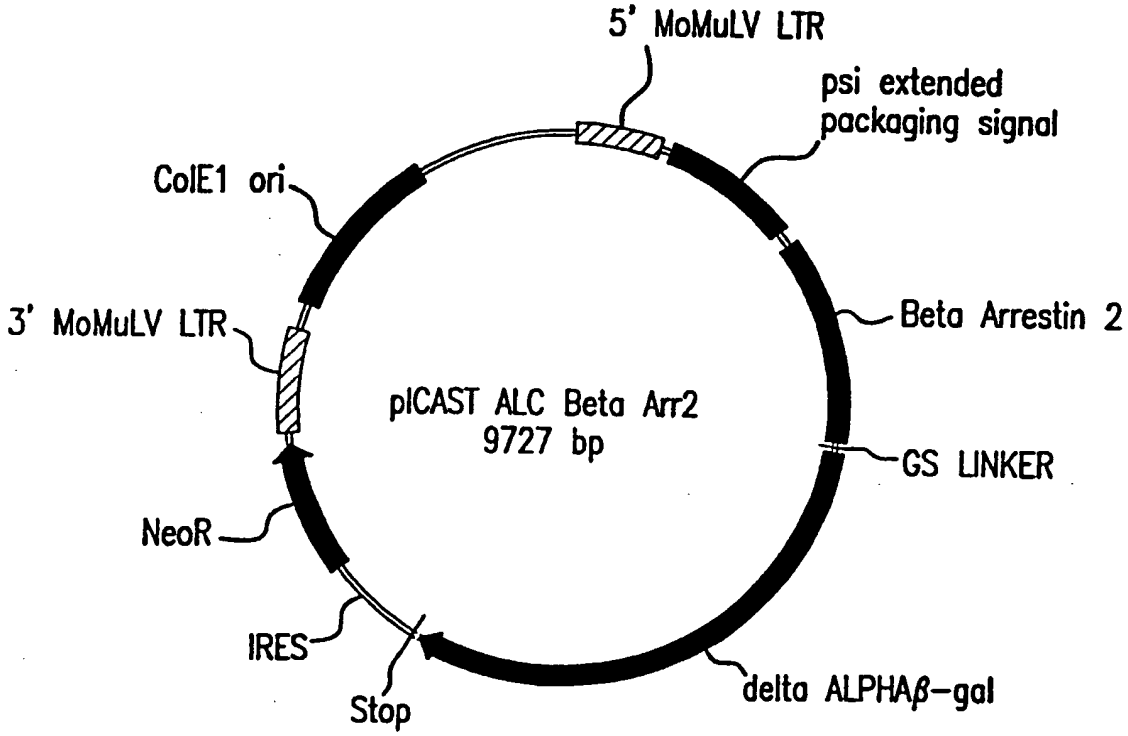
5' MoMuLV LTR
psi extended
packaging signal
ColE1 ori
3' MoMuLV LTR
Beta Arrestin 2
pICAST ALC Beta Arr2
9727 bp
GS LINKER
NeoR
IRES
Stop
delta ALPHAβ-gal

FIG.14

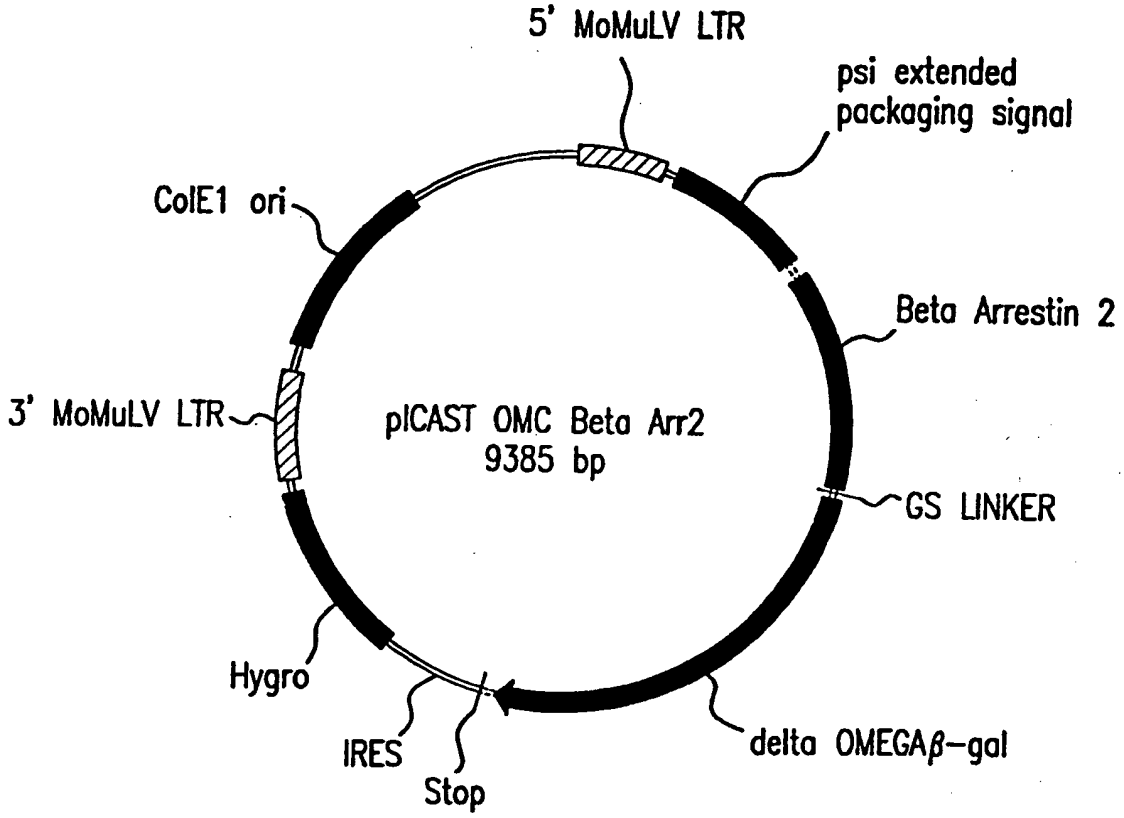
5' MoMuLV LTR
psi extended
packaging signal
ColE1 ori
Beta Arrestin 2
3' MoMuLV LTR
pICAST OMC Beta Arr2
9385 bp
GS LINKER
Hygro
IRES
Stop
delta OMEGAβ-gal

FIG.15

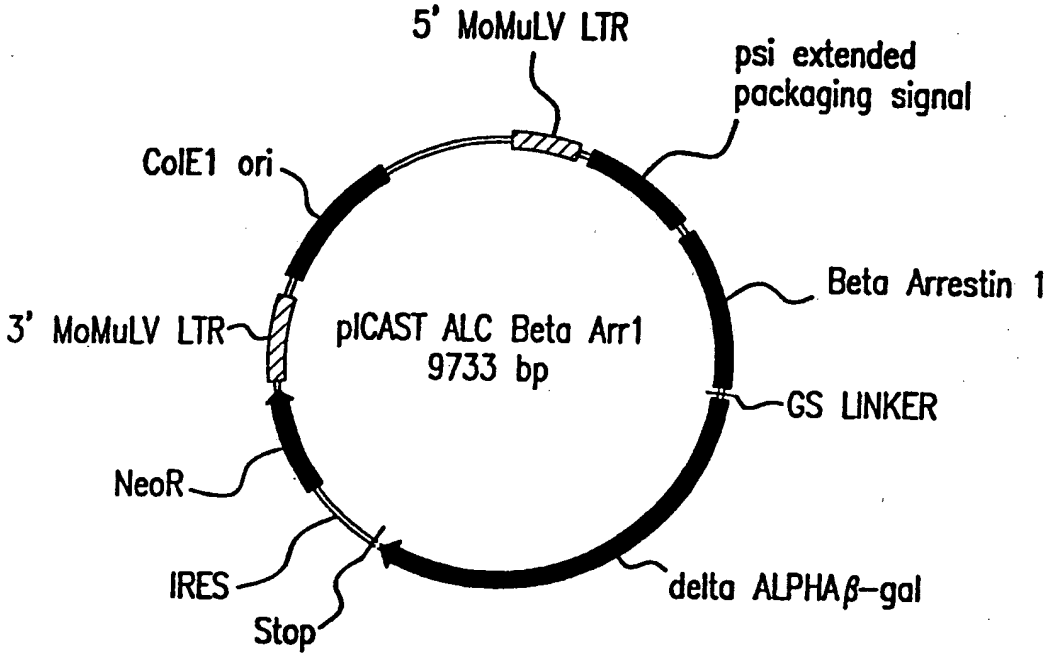
5' MoMuLV LTR
psi extended
packaging signal
ColE1 ori
Beta Arrestin 1
3' MoMuLV LTR
pICAST ALC Beta Arr1
9733 bp
GS LINKER
NeoR
IRES
Stop
delta ALPHAβ-gal

FIG.16

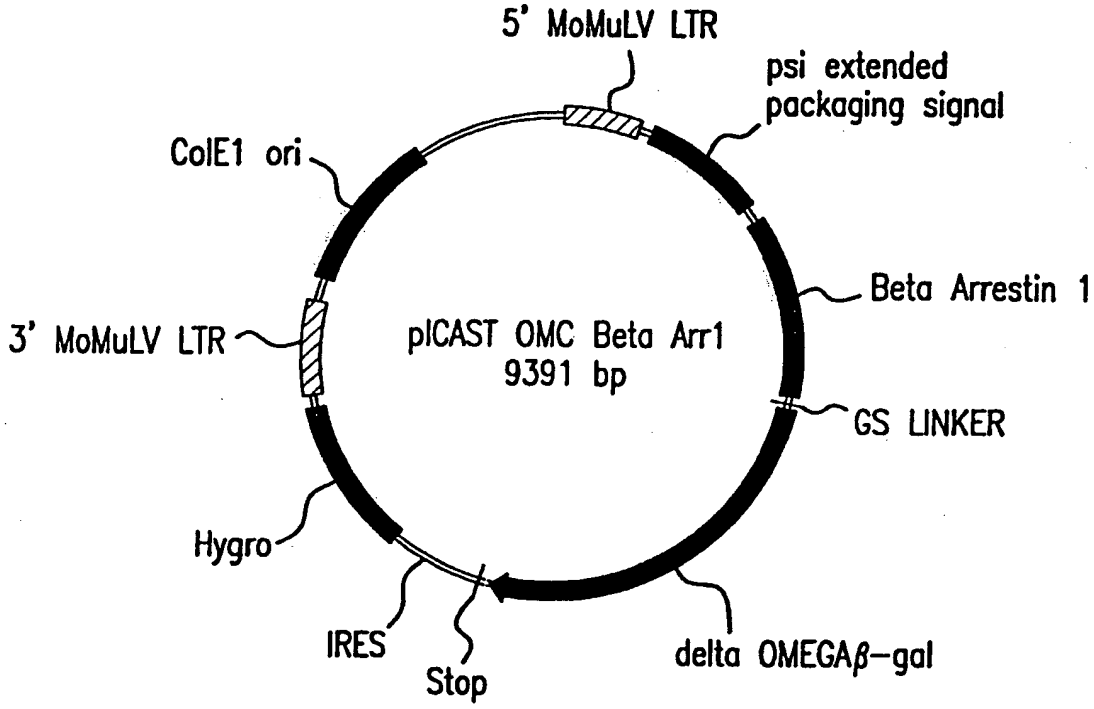
5' MoMuLV LTR
psi extended packaging signal
ColE1 ori
Beta Arrestin 1
3' MoMuLV LTR
pICAST OMC Beta Arr1
9391 bp
GS LINKER
Hygro
IRES
Stop
delta OMEGAβ–gal

FIG.17

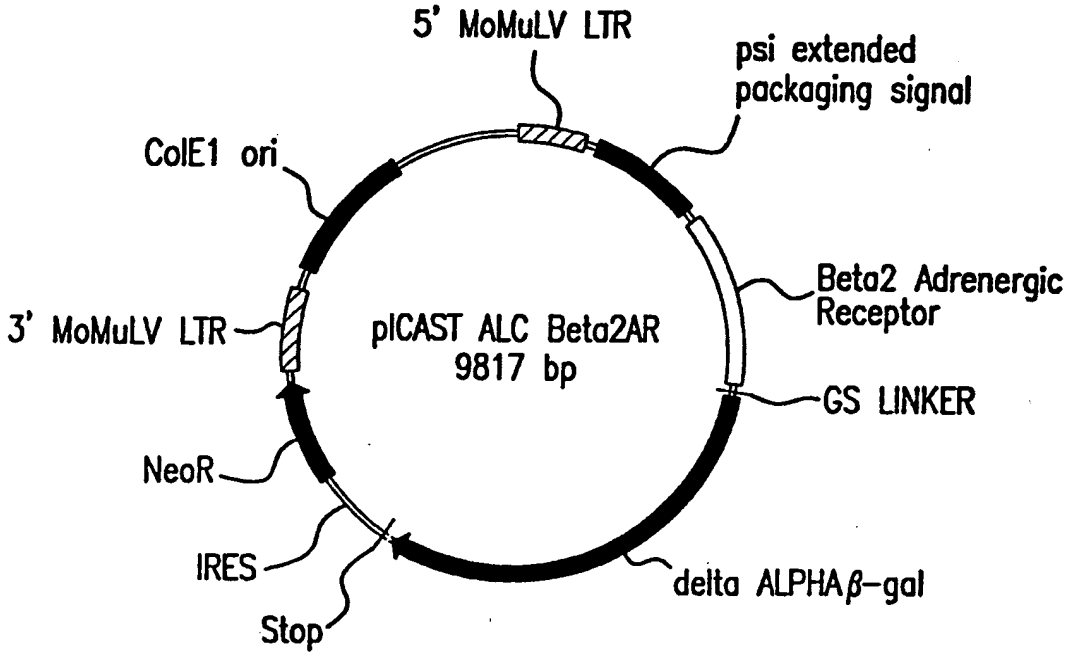
5' MoMuLV LTR
psi extended packaging signal
ColE1 ori
Beta2 Adrenergic Receptor
3' MoMuLV LTR
pICAST ALC Beta2AR
9817 bp
GS LINKER
NeoR
IRES
delta ALPHAβ-gal
Stop

FIG.18

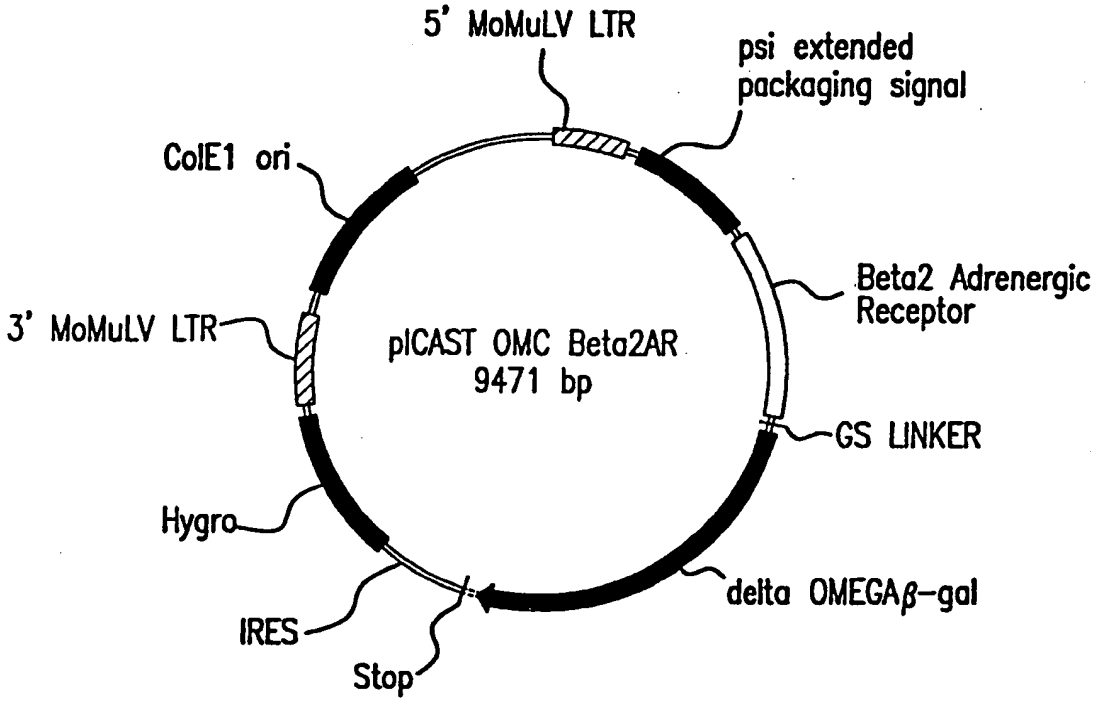
5' MoMuLV LTR
psi extended packaging signal
ColE1 ori
Beta2 Adrenergic Receptor
3' MoMuLV LTR
pICAST OMC Beta2AR
9471 bp
GS LINKER
Hygro
delta OMEGAβ-gal
IRES
Stop

FIG.19

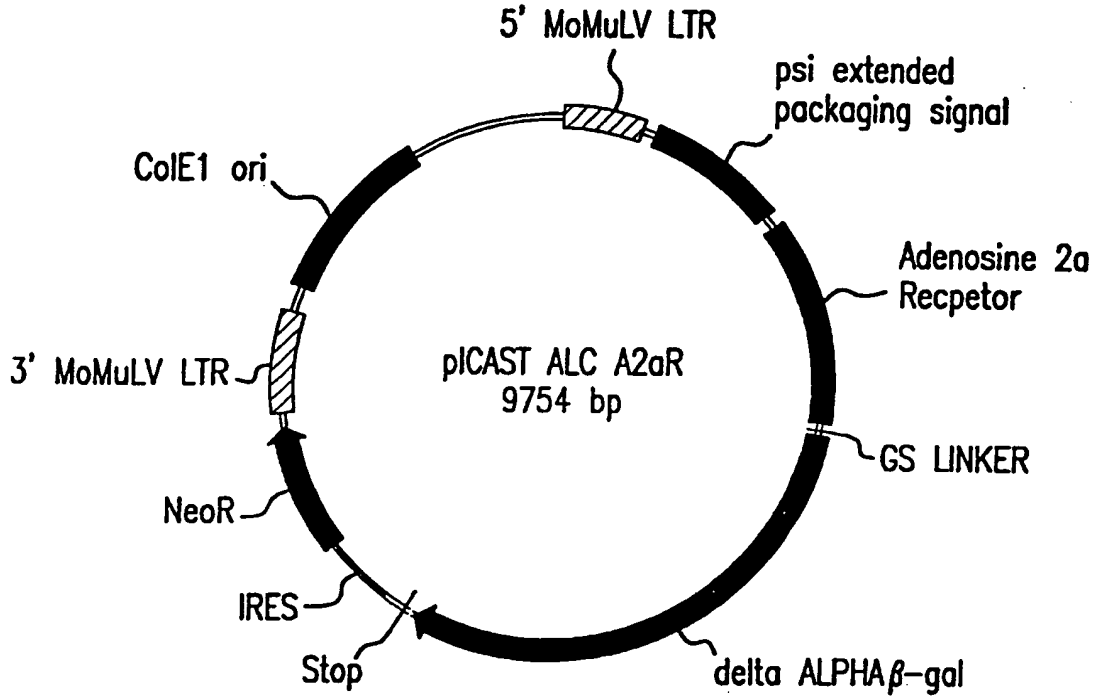
5' MoMuLV LTR
psi extended
packaging signal
ColE1 ori
Adenosine 2a
Recpetor
3' MoMuLV LTR
pICAST ALC A2aR
9754 bp
GS LINKER
NeoR
IRES
Stop
delta ALPHAβ-gal

FIG.20

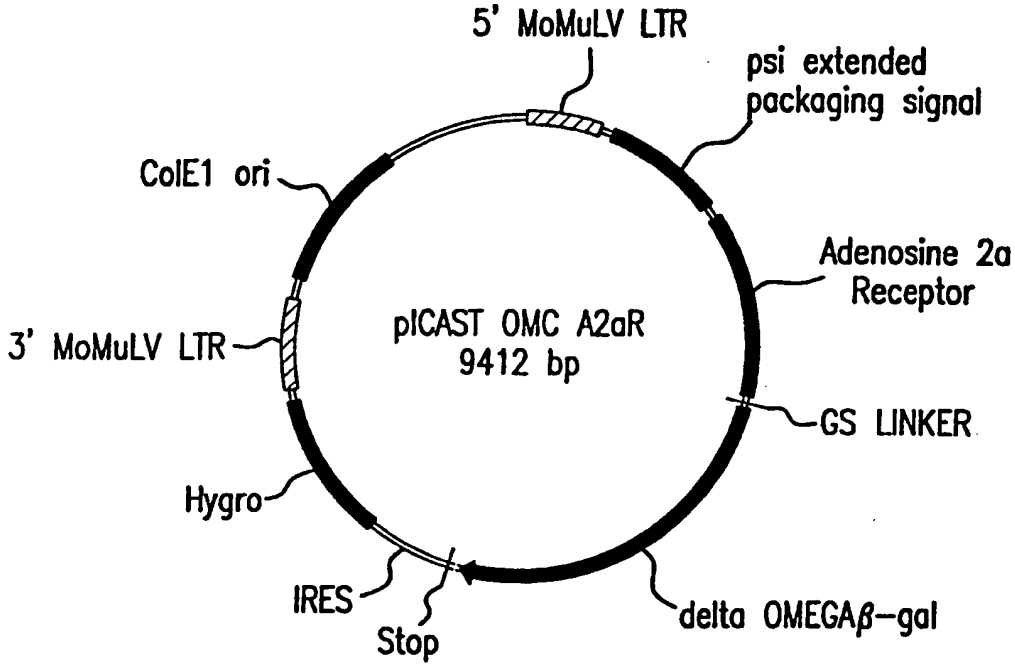
5' MoMuLV LTR
psi extended packaging signal
ColE1 ori
Adenosine 2a Receptor
3' MoMuLV LTR
pICAST OMC A2aR
9412 bp
GS LINKER
Hygro
IRES
Stop
delta OMEGAβ–gal

FIG.21

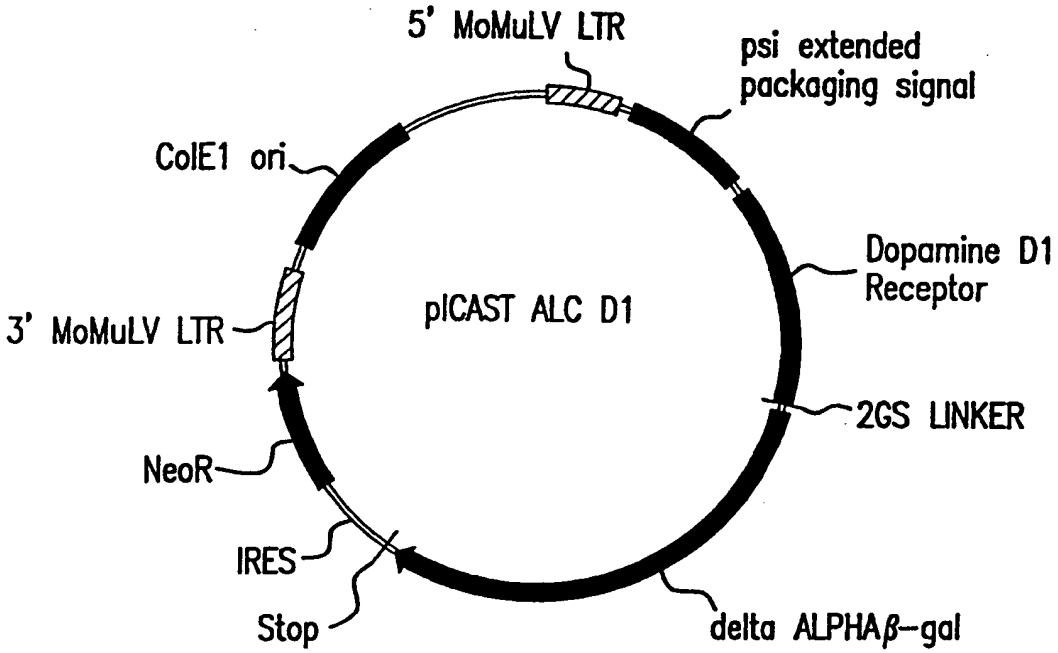
5' MoMuLV LTR
psi extended packaging signal
ColE1 ori
Dopamine D1 Receptor
pICAST ALC D1
3' MoMuLV LTR
2GS LINKER
NeoR
IRES
Stop
delta ALPHAβ-gal

FIG.22

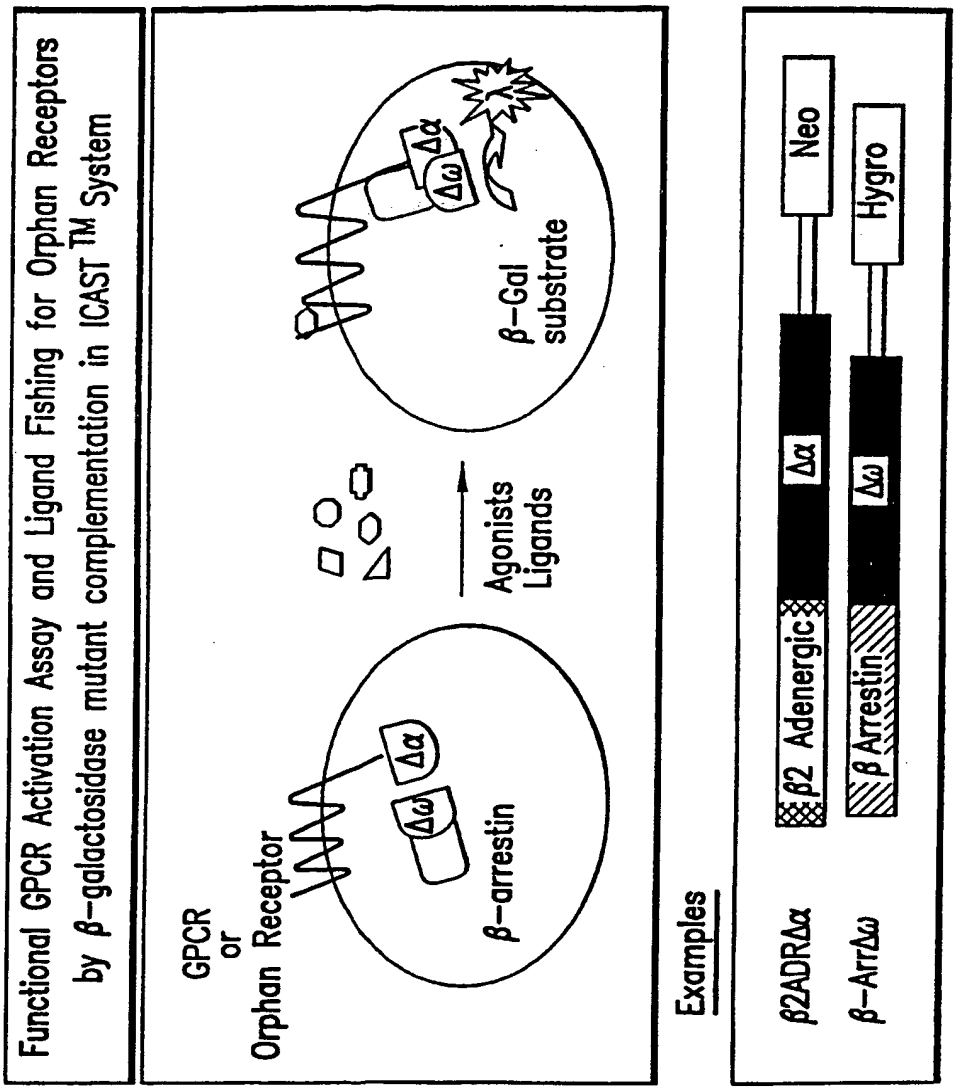
Functional GPCR Activation Assay and Ligand Fishing for Orphan Receptors
by β-galactosidase mutant complementation in ICAST™ System
GPCR
or
Orphan Receptor
Δω
Δα
β-arrestin
Agonists
Ligands
Δω
Δα
β-Gal
substrate
Examples
β2ADRΔα
β-ArrΔω
β2 Adenergic
Δα
Neo
β Arrestin
Δω
Hygro

FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/24043

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/52; C07H 21/04; C07K 14/00; C12N 15/12
US CL : 435/7.1, 7.2, 69.7; 436/501; 530/350; 536/23.4

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.2, 69.7; 436/501; 530/350; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: MEDLINE, USPATFULL
search terms: fusion#, hybrid#, chimer?, arrestin#, beta galactosidase#, protein-protein interaction

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,891,646 A (BARAK et al.) 06 April 1999, see entire document.	1-30
Y	BARAK et al. A beta-Arrestin/Green Fluorescent Protein Biosensor for Detecting G Protein-coupled Receptor Activation. The Journal of Biological Chemistry. 31 October 1997, Vol. 272, No. 44, pages 27497-27500, see entire document.	1-30
Y	ROSSI et al. Monitoring Protein-protein Interaction in Intact Eukaryotic Cells by beta-Galactosidase Complementation. Proceedings of the National Academy of Science. August 1997, Vol. 94, pages 8405-8410, see entire document.	1-30

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 NOVEMBER 2000	Date of mailing of the international search report 05 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN D. ULM Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*